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REFLEX SUMMATION IN THE IPSILATERAL SPINAL FLEXION REFLEX.

By J. C. ECCLES and C. S. SHERRINGTON.

(*From the Physiological Laboratory, Oxford.*)

Setschenov 1868(31), Stirling 1874(29), James Ward(30) and Exner(11) were the first investigators of reflex activity to appreciate the importance of summation of repetitive stimuli. From a consideration of their results Goldscheider(16) regarded each neurone as presenting a definite threshold (the neurone threshold) over which the excitation produced by afferent impulses had to rise, *e.g.* by summation, before a discharge of the neurone was produced. The features of reflex summation were also well shown in the scratch reflex(22, 23). By this time division into the sub-groups of spatial and temporal summation had been well established; it was recognized that both forms depended on the central overlap of the effects of the excitatory stimuli, the locus of summation probably being the synapse. Lapicque(17) confirmed the central location of reflex summation, for he found it to be greatly affected by heating or cooling the spinal cord, while similar treatment of the afferent nerve gave negative results.

The essential basis of the division(18, 19) of reflexes into two classes—*d'emblée* and recruiting—depends on the extent to which the central effect of an exciting impulse is influenced by those which precede it. In a typical *d'emblée* reflex, the spinal flexion reflex, a succession of afferent impulses is unable to raise above threshold any more neurones than is a single impulse.

The duration of the excitatory process in those neurones excited below neurone threshold by a single impulse must be so short that it has disappeared before the next impulse arrives, else summation would be present, and a series of impulses would bring in additional motor units. In a recruiting reflex, the decerebrate crossed extensor reflex, on the other hand, a single stimulus is often ineffectual, and, with each repetition of the stimulus, more and more neurones reach the neurone threshold. The central excitatory process must be of such duration that

each successive impulse is able to add its excitatory effect to that remaining from the previous impulses until the neurone threshold is attained.

Here is a conception which implies that the duration of the central excitatory state varies greatly for different types of reflex. The purpose of the present work is to attempt to study by a more direct method the time relations of the central excitatory process following the arrival of a single volley of excitatory impulses at the neurone.

METHOD.

The animal (cats with the exception of two dogs) having been anæsthetized, any spinal cord operation necessary (division of dorsal roots or section of cord) was performed, immediately followed by decerebration by the trephine method. In some experiments the animal had been made spinal with aseptic precautions 4 to 6 days previously. Following decerebration, all the muscles except the one to be investigated were immobilized by appropriate nerve and tendon section, and the skin was denervated. The importance of as complete a denervation as possible has so impressed us that we have latterly considerably complicated our technique by extradural division of almost all dorsal roots belonging to the segments of isolated spinal cord whose reactions we were investigating with the exception of those in the afferent path of the reflex. Muscle immobilization by tendon resection is much less satisfactory than denervation, so, with all limb muscles except psoas, denervation has been performed. Skin has also presented problems, for the traditional operation does not denervate the skin in the gluteal region, which has been rendered insensitive in some recent experiments by section of the dorsal divisions of the lower lumbar spinal nerves. The aim of this extensive preparation was to free, as far as possible, the investigated reflex responses from all extraneous complicating stimuli.

The mirror myograph employed in our earlier experiments had a frequency of 660 D.V. per second, and, at the usual magnification of about 600, it gave an image movement of 5 mm. for 10 g. More recently we have used a myograph of similar sensitivity but with a frequency of 1300 D.V. per second. Such high sensitivity is necessitated by the weakness of the twitches which we have had to record. In later work electromyographic records with a Cambridge string galvanometer have been combined with the myographic: the bright myographic image registers photographically on the less bright background from the galvanometer optical system. All observations are measurements from photographic records taken by a falling-plate camera.

Care has been taken to control the temperature of the preparation, and all exposed tissues have been kept moist by warm Ringer.

Reflexes have usually been elicited by single break induction shocks applied to nerves kept under constant conditions by fluid electrodes (6) by which the nerve was stimulated as it lay in defibrinated blood taken from the animal itself. Single shocks at various time intervals were obtained by an electromagnetically released pendulum which was

reliable to 0·1σ for intervals less than 16σ. A second excitatory stimulus has been used to sample the central condition at any particular interval after the first stimulus[1]. Under these conditions any difference in the response to the second stimulus depends on the central effects remaining from the first stimulus. To avoid complications produced by wave summation in the muscle (20, 15) by two stimuli following in quick succession, it was necessary to have the first stimulus at threshold intensity[2] (neurone threshold), and, for the purpose of further simplification, the second stimulus was also usually at threshold.

Results.

The first experiments using complex nerves (superficial peroneal, anterior tibial, internal saphenous) as afferents had given such complicated results that it became necessary to choose our afferent nerves and our muscle so as to simplify the analysis as much as possible. All afferents from skin must necessarily be composed of fibres from such widely differing receptors as those for pain, pressure, touch, and temperature. It, therefore, seemed better to employ afferent nerves from muscles. When both stimuli are applied to the same afferent, it is impossible to rely on recovery from the relative refractory period following the first until 6σ has elapsed. The period from simultaneity to this interval was found to be of such interest and importance that it was essential for the second stimulus to be applied to a different afferent from the first. It is advantageous to choose closely allied afferents, as the amount of central overlap (occlusion) between them is greatest (5, 10). In the most instructive experiments we have used the two nerves to gastrocnemius (one to the medial head and the other to the lateral head and soleus) as afferents reflexly exciting tibialis anticus.

I. *Tibialis anticus.*

The method of experiment was to find the threshold for reflex excitation of tibialis anticus by a break-shock given by one pendulum key to one nerve to gastrocnemius (central end of cut nerve) and then by the other key for the other nerve. The two shocks, one to each, are then given at various intervals from simultaneity to one or the other leading

[1] With the afferent nerves used the peripheral paths have been closely similar, *e.g.* two gastrocnemius nerves, dorsal digital nerves, or the two branches of peroneal used as pairs, so that the interval between the stimuli can be taken as equal to the interval between the arrival of the afferent volleys in the reflex centre.

[2] We have often used a strength of stimulus just above threshold, so that the smallest observable reflex response (1 to 2 g.) was obtained.

by such an interval that the preceding stimulus ceases to have any influence on that following. It is found that these two non-effective stimuli when combined in certain time sequences produce a reflex contraction of the muscle. Great care has been taken to exclude the following possible sources of this enhancement of the effect of one stimulus by the other.

(1) Physical effect of one electrical current on the other. The two secondary coils are placed about three metres apart at right angles to each other. The wiring of the primary and secondary circuits has been as straight as possible, and all wires are run as widely separated single core conductors. The effectiveness of these precautions has been shown by simultaneous breaking of both primary circuits with one secondary short-circuited, having exactly the same stimulating power as the shock from the unshort-circuited secondary alone.

(2) Spread of stimulus from one electrode to the other, or from either or both electrodes to the motor nerve, or to other afferents. The best safeguard against this danger was its ease of detection. It was shown[1] that the duration of effect of one break induction shock on motor nerve fibres was not longer than 0·5σ when the conditions of circuit, key, and coil were almost identical with those used in these experiments, so that the influence of the spread of one stimulus on the effectiveness of another could only last for this period. It has been very rare for the simultaneous stimulation of both afferents to differ in reflex effect by more than a slight amount from stimulation with either leading by 1σ, at which interval spread of stimulus could not be a complicating factor.

(3) A summational effect in the muscle itself whereby two or more stimuli in rapid succession give a larger effect than the sum of each individually (wave summation). Although apparently causing no reflex effect either stimulus alone may be reflexly exciting a few motor units whose twitch contraction is not registered by the myograph because (*a*) the inactive muscular mass exerts a damping effect, (*b*) the contracting muscle fibres are not initially taut owing to insufficient initial tension or to inequality of the tension distribution, (*c*) the frictional resistance of the myograph cannot be overcome by the very small torsional force. Effect (*b*) can be excluded because it has been found that further increase of the initial tension used (about 50 gr.) did not cause a subliminal stimulus to become liminal. The effects (*a*) and (*c*) are difficult to gauge, but, as electrical records show absence of action-

[1] The secondary circuit was broken by one pendulum key at various intervals after the breaking of the primary circuit by the other pendulum key.

currents when the myogram shows absence of contraction, it seems that the interference is not great. The weak contractions undoubtedly suffer more than the strong, and hence the degrees of facilitation which we have shown are somewhat augmented. Moreover, the reflex facilitation curves have never resembled "wave summation" curves, so it seems certain that the curves we have obtained, while modified by the above factors, are in essentials independent of them.

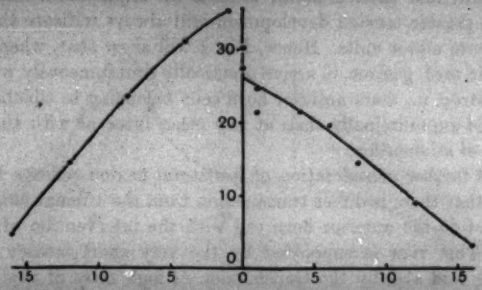

Fig. 1. Reflex responses of tibialis anticus to stimuli (alone just threshold) to med. gastroc. n. and lat. gastroc. n. at various intervals. Abscissæ = time in sigmata, ordinates = tension in g. To the right of zero is shown interval by which stimulus to lat. gastroc. n. is leading—to the left by which med. gastroc. n. is leading. (The response of the preparation altered somewhat between the observations on these two sides.) Each point plotted shows the tension developed at the indicated interval between the stimuli.

There remains the possibility of the facilitation of the central effect of one stimulus by that of the other. In Fig. 1 each point represents the tension developed by reflex contraction of tibialis anticus[1] in response to a just threshold stimulus of one nerve to gastrocnemius separated by the indicated interval from a just threshold stimulus to the other nerve to gastrocnemius (for convenience henceforth called med. gastroc. and lat. gastroc. nerves). It will be noticed that in this and other figures some of the points lie away from the curves which we have attempted to draw[2]. The degree of variability has been such that the

[1] The approximate proportion of the motor units of tibialis anticus brought into activity in any of the reflex twitches can be calculated on the assumption that the maximum motor twitch is about 400–500 g. for the type of animal used.

[2] Great care has been exercised, by random selection of intervals, to avoid any gradual change in the preparation affecting the shape of the curve. Usually at least one repetition of each observation has been made later in the series. All the observations of a consecutive series have been plotted in the curves shown.

actual shape of the smoothed curves can have little significance. We can only rely on such features as the position of optima and the degree of facilitation. The presence of "angles" in records from which the measurements have been made shows the influence of friction in the myograph bearing and warn against a too precise interpretation of the curves. Although the electrical responses of the muscle indicate that the reflex discharge is a single volley, a direct proportionality between tension development and excited motor units is an unjustifiable assumption. However, greater tension development will always indicate the excitation of more motor units. Hence, Fig. 1 will show that, when afferent impulses in med. gastroc. n. arrive practically simultaneously with those in lat. gastroc. n., more anterior horn cells belonging to tibialis anticus are excited supraliminally than at any other interval with these same strengths of stimulation.

In the further consideration of ipsilateral flexion reflexes it will be assumed that there is direct transmission from the afferent entering the dorsal root to the anterior horn cell with the intervention of only one synapse. This view is supported by the very short latency of these reflexes (13) and also by the transmission of high rates of rhythm from afferent to efferent (2, 4).

It is evident from Fig. 1 that the first stimulus though unable to excite any anterior horn cell sufficiently to cause it to discharge an impulse down its axis cylinder, nevertheless produces somewhere in the central path a state of raised excitability, for the second stimulus, which alone was able to excite no anterior horn cells, is now able to cause discharge from some when applied within a certain time interval from the first. One can think of the second stimulus as sampling the central condition remaining at various times after the first stimulus. As the stimuli are applied to different afferents which separately converge on the anterior horn cells, one would expect that, with simultaneous stimuli, there would be more central facilitation, for the central process being capable of summation cannot have a refractory period. Even if the central excitatory process took some time to develop, one would still anticipate that simultaneous arrival of impulses on the anterior horn cell would have an optimal effect, for the maximum central excitatory condition of each would be reached at the same time. But it is by no means usual to obtain such a simple facilitation curve as Fig. 1, curves such as Fig. 2 being more common. Here it will be noticed that there is a distinct optimum facilitation when the stimulus to med. gastroc. n. precedes that to lat. gastroc. n. by 12σ. When the stimuli are

applied in the reverse order, there is no sign of such an optimum. The complexity of the results is best indicated by a brief summary of the more successful experiments.

Exp. 21. xii. 28. Marked optimum with stimulus to med. gastroc. n. leading by 12σ (both stimuli threshold). When stimulus to med. gastroc. n. weakened and stimulus to lat. gastroc. n. increased to above threshold this optimum changed from 12σ interval to 6σ interval, though there was no marked falling away in response until the interval was greater than 12σ. No optimum was observed with stimulus to lat. gastroc. n. leading.

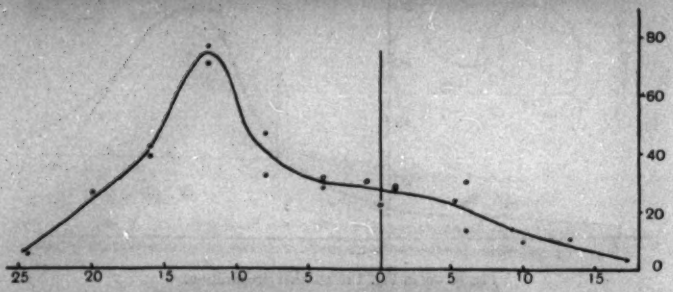

Fig. 2. As in Fig. 1 in a different preparation.

Exp. 7. i. 29. A well marked optimum with stimulus to lat. gastroc. n. leading by 12 to 14σ with a slight doubtful optimum at simultaneity.

Exp. 8. i. 29. At first there was an optimum with simultaneous stimuli. It soon changed and a well marked optimum with stimulus to lat. gastroc. n. leading by 13 to 14σ appeared (Fig. 3). Later the optimum at simultaneity returned and no trace of the optimum at 13 to 14σ interval remained.

Exp. 10. i. 29. A well marked optimum with simultaneous stimuli with no signs of optima at any other interval (Fig. 1).

Exp. 11. i. 29. This was the most instructive experiment and will be dealt with in more detail. Fig. 2 illustrates the curve obtained with both stimuli at threshold. All possible alterations in the strength of the stimuli failed to reveal an optimum with stimulus to lat. gastroc. n. leading, but an optimum at simultaneity was well marked under some conditions (Fig. 6).

Exp. 21. v. 19. Optimum at simultaneity.

Exp. 4. vi. 29. Three optima present: med. gastroc. n. stimulus leading by 12σ, simultaneity, and lat. gastroc. n. stimulus leading by 11σ.

To sum up: In three experiments an optimum occurred when stimulus to med. gastroc. n. was leading by 12σ (changed to 6σ in 1st exp.), in three others when stimulus to lat. gastroc. n. was leading by 11–14σ, and in six at simultaneity (one not well marked).

These results indicate that the central arrival of a volley of nerve

impulses in either the lat. gastroc. or med. gastroc. afferents can produce, in the anterior horn cells of tibialis anticus, two excitatory conditions with distinct time relations, one reaching a maximum almost immediately and the other after about 12σ. This is made especially clear in Exp. 8. i. 29, where one type of facilitation was entirely replaced by the other and then later the reverse change took place. Fig. 3 shows the

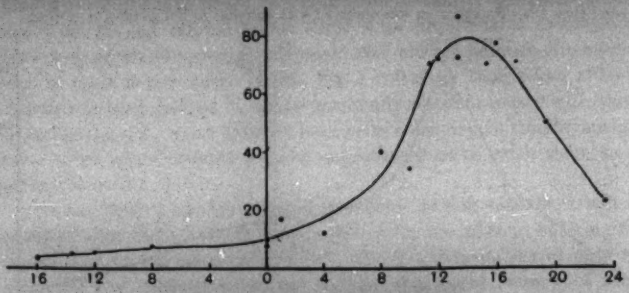

Fig. 3. As in Fig. 1 in another preparation.

definite optimum existing at 14σ, of which no trace could be found 2 hours before or 2 hours after, it being replaced by the optimum at simultaneity. Unfortunately the preparation was not under investigation during the transition period, but it seems likely that it was due to slight changes in the relative excitability of the nerve fibres causing an alteration in the nerve fibres stimulated.

After Fig. 2 (in Exp. 11. i. 29) had been obtained both stimuli were applied to the med. gastroc. n. Although the relative refractory period would interfere with the stimuli less than 6σ apart, still the optimum at 12σ should remain; but the curve shown in Fig. 4 was unexpectedly found. A return to the original observation showed that the type of curve with an optimum at 12σ was still being given. Thus different sampling afferents give evidence of different excitation after-effects following the first stimulus.

As each anterior horn cell will not be played upon in the same way or to the same extent by each afferent, and, doubtless, some anterior horn cells will only be played on by one afferent, this condition would be expected to some extent. But no such wide divergence as is here represented can be completely explained by such a consideration. In Fig. 4 the second stimulus is of equal intensity to the first, so that all

the anterior horn cells stimulated by the first will also be stimulated by the second. How is it, then, that no evidence is obtained of those anterior horn cells which sampling by lat. gastroc. n. shows to be most excitable 12σ after the stimulus to med. gastroc. n.? Fig. 5[1] will make the argument clearer. The area enclosed by the outer circle represents all the

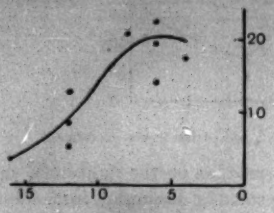

Fig. 4.

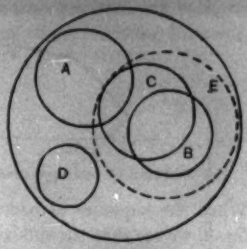

Fig. 5.

Fig. 4. As in left-hand side of Fig. 2 except that both stimuli were applied to med. gastroc. n. Registered immediately after Fig. 2.

Fig. 5. Diagram, explanation in text.

anterior horn cells of tibialis anticus and a small area *A* corresponds to those neurones on which the subliminal central excitatory effect of threshold stimulus to med. gastroc. n. is exerted. Area *B* represents the neurones on which a delayed effect is exerted by the same stimulus. This delayed effect reaches its maximum 12σ after the arrival of the stimulus. It must be understood that this is purely diagrammatic, and it is very unlikely that any strict topographical localization exists in the spinal cord. Areas *A* and *B* probably do not overlap, for, when the effect of a stimulus to med. gastroc. n. is sampled by a similar stimulus, there is no indication of an optimum at 12σ interval[2] (Fig. 4). In the same diagram area *C* could represent those neurones subliminally excited by the stimulus to lat. gastroc. n. There will be two distinct time processes involved. (*a*) That having an optimum at simultaneity and steadily diminishing as the interval is increased (overlap of *C* with *A*). (*b*) That having an optimum at 12σ (overlap of *C* with *B*).

[1] Areal representation conveniently depicts by overlap units common to two afferents, as evidenced by the occurrence in them of summation.

[2] The non-existence of the optimum at 12σ interval is not a certain proof of the absence of overlap of areas *A* and *B*. Complications are introduced if the delayed excitation is a rebound following the initial inhibition (see pp. 10, 14), for then the tendency to rebound in area *B* following the first stimulus would possibly be prevented by the initial inhibition of the second.

Though the curve of Fig. 2 does not seem to be composed of two distinct curves, several other curves obtained under similar conditions in that experiment showed a decided minimum at 4 to 8σ clearly separating two optima, *e.g.* Fig. 6, though there the conditions are not so constant.

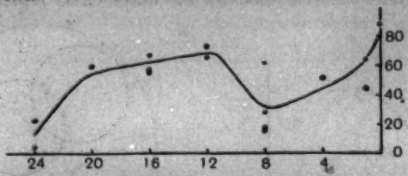

Fig. 6. Taken on same preparation as Fig. 2, but earlier in the experiment.

When the sequence of stimuli is reversed, as on the right-hand side of Fig. 2, we see that the reflex response steadily diminishes from simultaneity till it becomes almost negligible at an 18σ interval. Its simple form can easily be explained by the overlap of *C* and *A* already postulated to explain facilitation at intervals close to simultaneity. The delayed effect of any area *D* produced by stimulus to lat. gastroc. n. obviously does not overlap with the excitatory effect of med. gastroc. n. (area *A*).

When the central excitatory condition following the threshold stimulus to lat. gastroc. n. was sampled by a second similar stimulus, a curve of the type of Fig. 4 was obtained with an optimum at 6σ, values at intervals less than this probably being diminished by the influence of the relative refractory period of the afferent nerve. As no facilitation in the region of 12σ was obtained, it seems that the delayed effect of lat. gastroc. n. (if any) does not overlap with the excitatory, *i.e.* area *D* with area *C*. Such a condition would not be improbable, for an unphysiological act, such as electrical stimulation of an afferent nerve, is quite likely to excite afferent nerve fibres having different effects on the same anterior horn cell.

Denny-Brown and Liddell(8) found that the ipsilateral extension reflex in the fore-limb (supraspinatus) possessed a long latency—60σ; evidence showed that the delayed central excitation was really a rebound following a preliminary inhibition. To test whether a similar explanation would apply in our case, we intensified the sampling stimulus to lat. gastroc. n. so that it alone produced a moderate response. If inhibition were present one would expect that, when the med. gastroc. n. stimulation just preceded lat. gastroc. n., the reflex response would

be less than that to lat. gastroc. n. alone (Fig. 8). Such was not the case nor was there any sign of an optimum at 12σ interval. This is probably due to lat. gastroc. n. exciting above threshold all those neurones of area *B* (as shown by dotted circle *E*), so that the facilitation produced by their delayed excitation was now occluded. (With intermediate strengths of stimuli the optimum at 12σ emerges from the background excitation.) The presence of an inhibitory effect following immediately

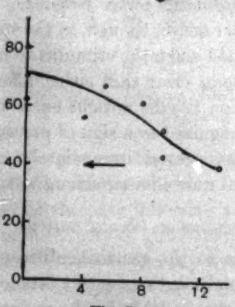

Fig. 7.

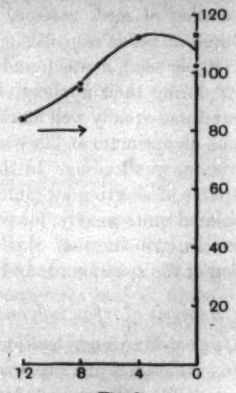

Fig. 8.

Fig. 7. As in right-hand side of Fig. 2 except that stimulus to med. gastroc. n. was now increased so that it produced a reflex twitch of 40 g., marked by arrow.

Fig. 8. As in left-hand side of Fig. 2 except that stimulus to lat. gastroc. n. was now increased so that alone it evoked a reflex twitch of 80 g., shown by arrow.

on the central arrival of the afferent volley from med. gastroc. n. and later rebounding as the delayed excitation, was not disproved by this negative evidence, for it was quite possible that the increase of stimulus to lat. gastroc. n. was able to produce a central excitation sufficiently strong to completely overcome any such inhibition. The existence of facilitation from simultaneity to about 12σ is evidence that the above threshold neurones for lat. gastroc. n. do not extend to the whole of area *A*, there being still an overlap of subliminal excitation corresponding to the original overlap of *C* and *A*.

The simple type of curve shown on the right side of Fig. 2 still persisted when the sampling stimulus to med. gastroc. n. was strengthened

(Fig. 7), showing that increase of area *A* still failed to overlap the hypothetical area *D*. That such an area can exist is shown by the results of Exps. 7. i. 29 and 8. i. 29, where optima with the stimulus to lat. gastroc. n. leading by 12 to 14σ are present (Fig. 3).

Evidence of the existence of rebound in tibialis anticus centre following stimulation of gastrocnemius afferents has been found in the only experiment (21. xii. 28) where the preparation was examined in the decerebrate condition before being made spinal. Even quite strong stimulation of med. gastroc. n. or lat. gastroc. n. with single shocks produced no reflex response in tibialis anticus, but, with tetanic stimulation (40 per sec.), it was found that rebound followed quite weak stimuli which during their application had produced no reflex response. The rebound was equally well marked with either nerve, though, in the spinal animal, an optimum at 12σ was only obtained when the stimulus to med. gastroc. n. was leading. In this case it seems clear that either afferent is capable of exerting an inhibitory effect on tibialis anticus even when stimulated quite weakly, for rebound is recognized as a sign of preceding inhibition. All trace of this rebound disappeared immediately after section of the spinal cord, and weak stimuli now elicited strong reflexes.

Analysis of reflex influence of gastrocnemius on tibialis anticus.

Denny-Brown(7) has found that taps to the gastrocnemius-soleus tendon sometimes produced twitches in tibialis anticus with a latency of just over 20σ. He suggested that this might possibly be the reciprocal accompaniment of the silent period in the extensor muscle. We have confirmed these observations in the de-afferented spinal tibialis anticus and have found that the latent period has been remarkably constant, 19 to 21σ in the cat and 24 to 26σ in the dog (medium size).

In all preparations we have observed that the tibialis anticus twitch has been more closely related to the strength of the tap than to the size of the gastrocnemius-soleus jerk. A typical delayed twitch in tibialis anticus has even been found where there was no gastrocnemius-soleus response following the tap. After completely cutting all the motor fibres to gastrocnemius and soleus (by severing the appropriate anterior roots, the dorsal roots remaining intact), it has been found that tapping the gastrocnemius tendon still evokes a twitch in tibialis anticus. It therefore seems certain that part, at least, of the tibialis anticus twitch must be a direct reflex response to the tension rise following the tap to gastrocnemius tendon.

If excitation of the muscle-spindles of gastrocnemius-soleus were

responsible for the tibialis anticus twitch, then stimulation of the anterior roots of the spinal nerves supplying gastrocnemius-soleus should also produce reflex twitches in tibialis anticus. Moreover, the latency of the response should be less than the twitches following taps to gastrocnemius tendon as the path is now shorter (Fig. 9). Thus from *A* (tendon-

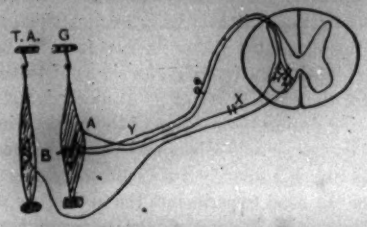

Fig. 9. Diagram of reflex paths connecting gastrocnemius (*G*) to tibialis anticus (*T.A.*). See text.

organ) to gastrocnemius anterior horn cell, to *B* (muscle-spindle) back to tibialis anticus anterior horn cell and then out to tibialis anticus would be the path following a tap to gastrocnemius tendon, while, when the motor fibres of gastrocnemius-soleus muscle were stimulated at *X*, the path would be directly from *X* to *B* and then as before. The 8th post-thoracic anterior root usually contains no motor fibres for tibialis anticus, and, as it supplies at least 30 p.c. of those to gastrocnemius-soleus, it is the root we have chosen for stimulation. In two preparations a direct motor response in tibialis anticus (as shown by a latency of 4 to 6σ in the electrical and mechanical effects) spoilt the experiment. In four other experiments motor twitches in gastrocnemius-soleus were unaccompanied by any contraction in tibialis anticus, though less powerful contractions of gastrocnemius-soleus in response to tendon taps were regularly associated with twitches in tibialis anticus. This result is difficult to reconcile with the view that muscle-spindle excitation is responsible for the tibialis anticus twitch.

In only one experiment was there a twitch in tibialis anticus associated with the gastrocnemius-soleus contraction following 8th anterior root stimulation (only gastrocnemius-soleus contracted as the motor nerves of all other muscles supplied by 8th anterior root were cut). Plate-fig. 1 shows the long latency (greater than 40σ) and repetitive nature of the tibialis anticus response. In this preparation we have two reflexes produced in tibialis anticus from gastrocnemius.

(1) A tap to gastrocnemius tendon evokes a twitch in tibialis anticus with a latent period of 19–20σ.

(2) Following a twitch in gastrocnemius-soleus there is a repetitive response in tibialis anticus with a latent period of just over 40σ.

As we have already seen that the tap to gastrocnemius tendon can reflexly excite tibialis anticus, it is reasonable to suggest that the tension produced by the twitch reflexly excites tibialis anticus in the second case. In the former the afferent nerves from the tension receptors would be stimulated within a fraction of a sigma of the onset of the tap, while in the latter the extra delay of 20 or more sigmata would be due to the time elapsing during the passage of the nervous impulse from the anterior root to gastrocnemius-soleus and the subsequent development of active tension to an intensity sufficient to excite the appropriate tension receptors. In most experiments the active rise of tension is not sufficient to evoke any tibialis anticus reflex response, for it is much more gradual in onset than the passive tension produced by the tap. The comparatively long-continued rise of active tension in the motor twitch would explain the repetitive nature of the reflex response under these conditions.

It was surprising to find that, when no resistance was offered to the contractions of gastrocnemius-soleus, there was still a reflex in tibialis anticus which was, however, much diminished (4 a). It seems to show that tension receptors are stimulated by a muscular twitch even when there is no external tension development.

The latent period of the tendon jerk in gastrocnemius averages about 10σ, and of this interval about 8σ can be accounted for in the peripheral path. For the tibialis anticus reflex the afferent path is the same as for the gastrocnemius jerk, and the efferent path is not greatly different. Why, then, is there a latency of 10σ longer, leaving a central time of 12σ to be accounted for? It is here that one calls to mind the frequency of an optimal facilitation existing at an interval of 12σ in the facilitating effect of one gastrocnemius afferent on the other. At this interval of 12σ after the arrival of the afferent volley, the central excitatory state is at its maximum, and consequently the discharge down the axis cylinder is most likely to occur then. The presumption is strong that the tension afferents of gastrocnemius-soleus are responsible for the optimal facilitation at 12σ.

What is the reason for this long delay? In the ipsilateral extension reflex it has been shown (8) that the long latency is due to the twitch really being a rebound following an inhibition; it is possible that the same explanation also applies to this case.

Plate-fig. 1. Reflex contraction of tibialis anticus in response to the muscular contraction of gastrocnemius-soleus evoked by stimulation of 8th post-thoracic anterior root. m_1 = myogram of gastrocnemius-soleus [pulling downwards (off the field at bottom of figure)—tension scale to left]; m_2 = myogram of tibialis anticus (pulling upwards—tension scale to right). The slight early fall in base line is due to give of fixation of limb caused by the strong gastrocnemius-soleus twitch. s = signal showing time of break-shock to 8th anterior root. g = string-galvanometer record—leads in tibialis anticus. The early action-current is due to spread of the strong action-current from the motor twitch in gastrocnemius-soleus. The delayed action-current shows the response of tibialis anticus with a latent period of about 40σ. Time between vertical lines = 20σ.

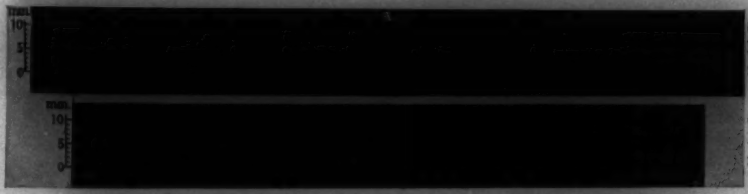

Plate-fig. 2. A record of response of tibialis anticus (de-afferented and with motor roots largely cut down) to a single stimulus to the ipsilateral sciatic nerve as shown by signal *S*. *m* shows myograph and *E* electrical record. From a chronic (8 months) spinal dog.

The development of central excitatory state in the anterior horn cells of tibialis anticus following a twitch in the lateral head of gastrocnemius and soleus has been investigated by sampling with a just liminal stimulus applied to the central end of the cut med. gastroc. n., *i.e.* by a technique similar to that used in investigating the central excitatory state following a nerve stimulus. Fig. 10 shows that there

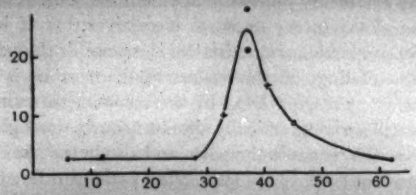

Fig. 10. As in previous graphs except that interval is now between first stimulus which is applied to the 8th anterior root, and the second stimulus which is applied to central end of cut med. gastroc. n. The tensions indicated for the two points marked by crosses are only approximate as they are not measurements of photographic records, but only values obtained by inspection.

was no sign of facilitation until the interval was greater than 28σ. The optimal facilitation occurred at 37σ interval; the effect then declined, and was over by 60σ. If this optimum at 37σ corresponds to the optimum at 12σ interval when one stimulus is applied to one gastroc. nerve and one to the other, there is an interval of 25σ between the stimulus at *X* (Fig. 9) and the arrival of the ensuing afferent nerve impulse at a point *Y* corresponding to the point where the stimulus is applied to the other gastrocnemius afferent. As the total latency of the tibialis anticus contraction was between 40 and 46σ, there is still an interval of about 18σ to be accounted for. This would correspond with a latency of 19 to 20σ in the tibialis anticus response to a tap to gastrocnemius tendon, and so support the view of the identity of the two reflexes. Fig. 10 bears a close resemblance to the delayed optimum facilitation curves of Figs. 2 and 3.

We have not succeeded in identifying the type of afferent fibre from gastrocnemius which is responsible for the immediate increase in central excitatory state, *e.g.* Fig. 1. It is not likely to be the muscle-spindle afferent, for the excitation of muscle-spindles from the anterior root does not seem able to produce reflexly any tibialis anticus contraction.

The time relations of the facilitation process distinguish between two types of central excitatory state.

(*a*) *The "direct" central excitatory state.* The excitatory effect on the individual anterior horn cells of tibialis anticus produced by a just threshold stimulus applied to one of the gastrocnemius nerves, *e.g.* area *A* (Fig. 5), will show all degrees of gradation from the smallest quantum of excitation to a condition of excitation just short of that where the cell discharges an impulse down its axis cylinder (neurone threshold). The intensity of the excitatory process in any neurone is certainly dependent on the number of excitatory impulses it receives, but it is not known whether a direct proportionality exists, for it is possible that the excitatory effect from some endings on the surface of the neurone is greater than that from others (*e.g.* (25), p. 524). In the response to a single stimulus these impulses will arrive practically simultaneously—a slight spread will be occasioned by differences in the path, and also in the speed of propagation, but it does not seem likely that delay paths can be of much significance in a cord sectioned in the upper lumbar region. The method of sampling used here necessarily only gives evidence of the existence of this subliminal excitatory state when the additional excitation of the sampling stimulus raises the excitatory level above threshold. Since it is found that, when both stimuli are applied together (and therefore reach the neurones in question practically simultaneously, for the central and peripheral paths are almost identical), the above-threshold level is attained in the most neurones, and the greater the interval between the stimuli, the fewer the anterior horn cells discharging, it must be concluded that the excitatory level produced by the first stimulus reaches its maximum with great rapidity (also shown by the brevity of the reduced reflex time of the flexion reflex) and then gradually declines. The lessened degree of facilitation at increasing intervals merely indicates that fewer neurones can now be raised above threshold by the second stimulus, and hence shows that, in the individual neurones, the excitatory state following the first stimulus is decreasing. The facilitation curves which we have obtained are only indirect statistical indicators of changes in the central excitatory state in the individual neurone. Though Fig. 1 shows practically no facilitation at 16σ, it does not indicate that the excitatory state produced by the first stimulus has then completely disappeared, for in those neurones where it still persists the sampling excitation may not be of sufficient intensity to enable the summated effect to reach threshold. It seems very probable that the central excitatory state persists longest in those neurones where it is initially most intense. A duration of 8 to 10σ usually obtains a few hours after spinal transection. This often increases to 20 or more sigmata

in the course of 24 hours. The lengthening with the longer period of recovery is probably a manifestation of the passing off of spinal shock. A duration of 20σ seems somewhat surprising for a subliminal excitation in a spinal flexor reflex, and doubtless with stronger excitation the duration is much longer, thus giving the explanation of the after-discharge following a single stimulus (24) and of the secondary waves in a tetanic response (1, 4).

Plate-fig. 2 is an extreme example of the long continued repetitive response in an ipsilateral flexor reflex. The spinal cord was transected 8 months previously and immediately before the experiment the muscle was completely de-afferented and most of its motor fibres were cut by almost complete division of the anterior roots supplying it. At first there are indications of action-currents in more than one motor unit, but soon one type of action-current alone remains, the intervals between successive firings increasing until an abrupt stoppage takes place. The view that one unit was responsible for these action-currents was strengthened by finding that alterations in the positions of the leads changed their shape without altering their identity in any particular record.

(*b*) *The delayed central excitatory state.* Usually its rise and decline are fairly abrupt. Though remarkably constant in its optimum time, it often shows a rather large degree of irregularity in tension development, *i.e.* in the number of discharging neurones. No differences other than degree of tension development can be seen between the myographic curves obtained at simultaneity of both stimuli and those with the optimum interval of 12σ. The delayed curve uncomplicated by the excitatory curve is shown in Fig. 10 where 25σ is equivalent to zero time (*v.s.* p. 15). It will be seen that, for at least 3σ later, there is no increase in the central excitatory state. This shows that, here, the initial central effect of the afferent impulses is not excitation and suggests that the delayed excitatory state may be a rebound following a short preliminary inhibition, though such an inhibition could not be demonstrated directly (*v.s.* pp. 10, 11). A rebound in 12σ is admittedly earlier than any yet described, but one must bear in mind that it is in a flexor muscle while other rebounds of this type have been in extensors.

II. *Semitendinosus.*

Various pairs of nerves have been used as afferents for reflexly exciting semitendinosus. As before, threshold stimuli were usually employed.

Spinal semitendinosus. (*a*) Superficial peroneal and anterior tibial nerves when used as afferents gave reliable results on three occasions.

Exp. 22. i. 29. There was a definite optimum when the stimulus to anterior tibial nerve preceded that to superficial peroneal nerve by 4σ (Fig. 11). The dip towards simultaneity

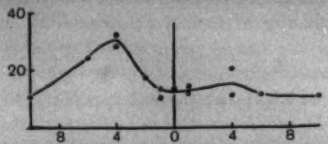

Fig. 11. Reflex responses of spinal semitendinosus to stimuli (just threshold) to anterior tibial and superficial peroneal nerves. Abscissæ show stimulus interval in sigmata. To the left of zero anterior tibial is leading, to the right superficial peroneal.

is curious, and indicates that, when the stimuli arrive at the anterior horn cell together, they have less effect than when separated by a small interval. When the stimuli were employed in reverse time relationship, there was one doubtful value giving a slight rise at 4σ, but it was not confirmed by repetition. When the stimuli to either or both nerves were increased, with anterior tibial leading there was still the same optimal interval of 4σ.

Exp. 17. xii. 28. In this experiment, 1 to 2 hours after cutting the spinal cord, there was a definite optimum with stimulus to anterior tibial nerve leading by 2 to 3σ. With simultaneous stimuli the degree of facilitation was quite small.

Exp. 13. iii. 29. Spinal dog, cord cut 6 days previously. The optimum occurs with stimulus to anterior tibial nerve leading by 2σ (Fig. 12). It agrees well with the previous

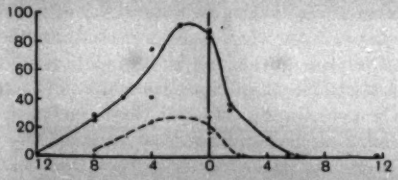

Fig. 12. As in Fig. 11, but in a chronic spinal dog (cord cut 11 days previously). To the right of zero superficial peroneal is leading, to the left anterior tibial. The dotted record and the crosses show the curve when the stimulus strength was weakened.

experiment in the short duration of facilitation existing when the stimulus to superficial peroneal nerve was leading. This curve was essentially unaltered by weakening both the stimuli. A definite second optimum was later found with anterior tibial stimulus leading by 16 to 20σ separated from the original optimum by a minimum of 14σ.

There is general agreement in there being a much greater and more prolonged facilitation when the stimulus to anterior tibial nerve precedes that to superficial peroneal, and this peculiarity is independent of the strengths of stimuli employed. The significance of this variation is

unknown, but it shows definitely that the afferents employed have complex central effects. These are to be expected from afferents having nerve fibres from both muscle and skin. The delayed rise in the last experiment again suggests rebound.

(*b*) In the hope of simplifying the complex conditions of the experiment, it was decided to try the effect of two pure skin nerves on each other. After dissecting up and cutting short the muscular branch of superficial peroneal, the remaining pure skin afferent nerve was split. It was easy to do this in all but one exceptional case, for, on incising the sheath, the four dorsal digital nerves were found lying discrete each from the others. Usually we separated the lateral two from the medial two.

Exp. 5. ii. 29. About 3 hours after section of the spinal cord there was a small degree of facilitation with simultaneous stimuli which diminished rapidly as the interval between the stimuli was increased. This is a type of early shock curve which we have found with many different afferents and with both semitendinosus and tibialis anticus as test muscles. Three hours later a totally different curve (Fig. 13) was obtained with similar strengths of

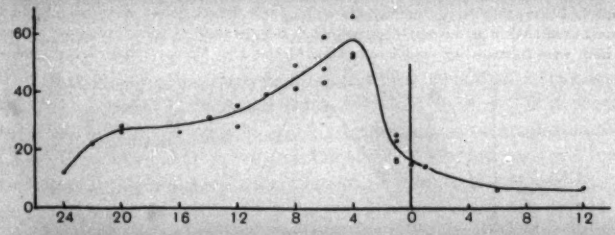

Fig. 13. Reflex responses of spinal semitendinosus to stimuli (just threshold) to lateral and medial divisions of superficial peroneal nerve, *i.e.* dorsal digital nerves. To the left of zero medial dorsal digital division is leading, to the right the lateral division.

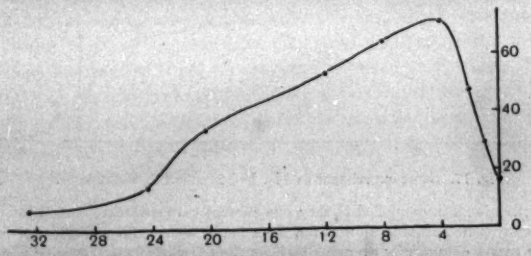

Fig. 14. As in Fig. 13, but stimulus to lateral dorsal digital increased so that just above threshold. Curve shows results with only medial dorsal digital division leading.

stimuli. The abrupt fall towards simultaneity from the optimum at 4σ is remarkable and resembles the curve when anterior tibial and superficial peroneal nerves were used as afferents. (Observations taken a little later confirmed the curve between 4σ and 1σ.) There is practically no facilitation when the stimulus to the lateral division leads by more

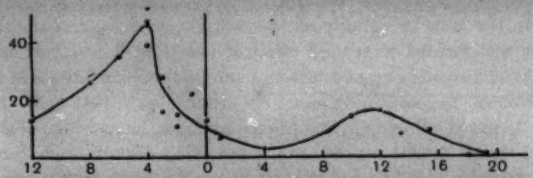

Fig. 15. As in Fig. 13, but stimulus to medial dorsal digital increased so that just above threshold, lateral dorsal digital stimulus being considerably below threshold. There is here an additional optimum when stimulus to lateral dorsal digital leads by about 12σ.

than 6σ. With the stimulus to the medial division leading there is considerable facilitation even at 20σ interval, beyond which an abrupt falling off occurs. Till the end of the experiment (3 hours) this very characteristic and grossly asymmetrical curve was found to remain unaltered in its essential features, when the strengths of stimuli to either afferent or both were increased or diminished (Figs. 14 and 15). Moreover the curves obtained

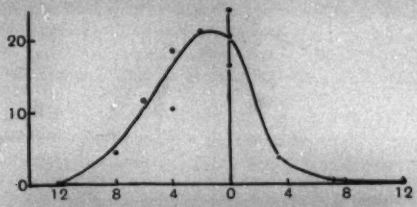

Fig. 16. As in Fig. 13, but in another preparation (about 2 hours after cutting of spinal cord). Just threshold stimuli.

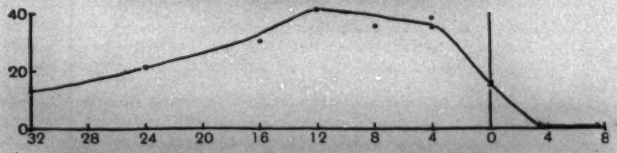

Fig. 17. Same experiment as Fig. 16 but about 2½ hours later.

when both stimuli were applied to the same afferent gave evidence, that, if the influence of refractory period could be allowed for, there would be essential similarity between them and the curves obtained by sampling each by the other afferent. When the effect of the stimulus to the lateral division was sampled by a similar stimulus, there were practically no signs of facilitation at any interval.

Exp. 11. ii. 29. About 2 hours after spinal section there was an early shock curve (Fig. 16) similar to that in the previous experiment; 2 hours later a curve (Fig. 17) similar to

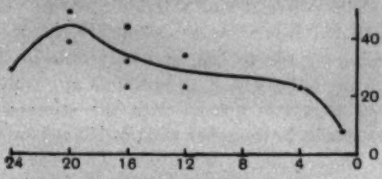

Fig. 18. About 1 hour later than Fig. 17. Only curve with medial division leading shown.

Fig. 13 was obtained, but with further continuation of the experiment the optimum was found to extend further out until it was at 20σ (Fig. 18). There was still a considerable degree of facilitation at 4σ which dropped sharply to simultaneity.

Exp. 12. ii. 29. A well developed optimum was present when the stimulus to the lateral division preceded that to the medial division by 8σ. The identity of the nerve bundles split was, however, not quite certain owing to a twist in the nerve.

The sudden diminution in facilitation which is obtained so frequently when the interval is decreased from 4σ to 0 is puzzling. Is it possible that two stimuli arriving in quick succession have a greater exciting power than when they arrive simultaneously? The time interval seems too short to be explained as a rebound. Whatever the process is, it is depressed after spinal transection, for it usually does not appear till 5 or 6 hours have elapsed. The limit for the demonstration of facilitation is usually 20 to 30σ. As yet no analysis, such as that attempted for the experiments on tibialis anticus, is possible, but it seems likely that the early shock curve is the manifestation of the simplest type of response, and that more complex conditions arise as spinal shock disappears.

A series of other experiments on spinal semitendinosus served to show that facilitation could be obtained between such widely different afferents (*i.e.* muscle or skin segmentally widely apart) as nerve to vastus lateralis and superficial peroneal (with its muscular branch destroyed), or internal saphenous and superficial peroneal.

Decerebrate semitendinosus. It was on semitendinosus in the decerebrate animal that we first started our investigations on the time relations of the excitatory process. In our first experiments both stimuli were applied to the superficial peroneal nerve, and therefore suffer from the interference of relative refractory period at intervals less than 6σ. Both, however, showed curves with a definite optimum which in one case was

at 12σ interval and in the other from 13σ to 20σ according to the strength of the stimulus.

When superficial peroneal and anterior tibial nerves were used as afferents, complicated results were obtained, *e.g.*, optimum intervals with either leading by 10σ to 14σ, and only once in the four experiments was there any indication of an optimum at simultaneity. In one experiment there were two optima with the stimulus to superficial peroneal leading, one at 2σ the other at 6σ to 10σ separated by a definite minimum at 4σ. The complexity of the central conditions is indicated by the compound nature of the time relations characterizing the central disturbance following a single afferent volley. One of the complicating factors is the inhibitory influence exerted on the flexors by the higher centres; this gradually increases after decerebration until often the flexion reflex is inelicitable. An immediate fall in reflex threshold follows abolition of this influence by section of the spinal cord (12, 20, 28). In the decerebrate animal the high threshold of the reflex response to a single stimulus, combined with a marked facilitation 10σ to 20σ after an ineffectual stimulus, can certainly be correlated with the recruiting nature of the reflex response of semitendinosus, for each preceding stimulus would facilitate the following until threshold was attained.

Besides causing an abrupt fall in threshold, spinal transection alters the form of the facilitation curve, but it is not certain how much this can be explained by the great difference in the strength of the stimuli used. In Exp. 4. xii. 28 a curve (Fig. 19) was obtained with a stimulus

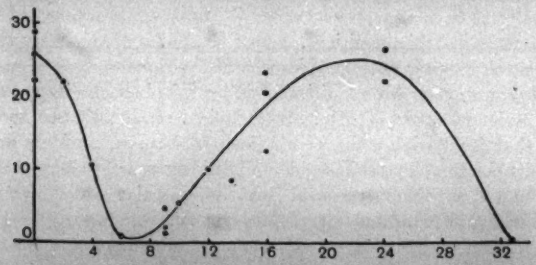

Fig. 19. Decerebrate semitendinosus responding to stimulus to superficial peroneal nerve followed by one to internal saphenous nerve. (Both stimuli below threshold.)

to superficial peroneal followed by one to internal saphenous (both stimuli at 12 cm. coil distance, either alone just threshold). When the

cord was transected, the threshold fell to 30 cm. coil distance for superficial peroneal nerve and to 26 cm. for internal saphenous. There was no sign of the previous optimum at 15σ to 25σ, there being only the typical early shock curve (Fig. 20). Previously to the cord section a

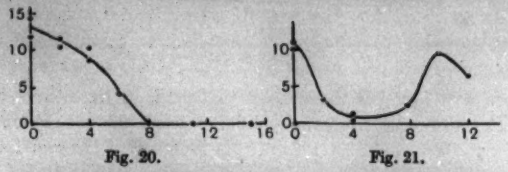

Fig. 20. Fig. 21.

Fig. 20. Same as Fig. 19, but spinal cord cut about ½ hour previously; stimuli just threshold, but much weaker than for Fig. 19.

Fig. 21. As in Fig. 20 but just before cord cut. The stimulus to superficial peroneal is the same strength as that used in Fig. 20.

curve had been obtained with the stimulus strength of superficial peroneal at 30 cm. coil distance (Fig. 21), and though then the second optimum was at 10σ, the shape of the curve was essentially similar to Fig. 19. Moreover, in the spinal condition, increase of the stimulus to internal saphenous nerve to a strength well above threshold (26 cm.) failed to show any sign of the second optimum. In this case it seems certain that spinal transection had removed the influence responsible for the second optimum.

The frequency of optima at intervals of about 12σ has been a feature with decerebrate semitendinosus, and raises the question of the essential similarity to the rebound phenomena in tibialis anticus. Unfortunately the mixed character of the afferents has rendered impossible any analysis of even the fragmentary character of that forthcoming in the case of tibialis anticus.

The following experiment was designed to distinguish between the threshold of the afferent nerve and the threshold of a reflex evoked by stimulation of it. Posterior tibial nerve was split for a considerable distance into two divisions *M* and *L*, the median and lateral plantar nerves; the calcanean branch was dissected up and cut short. It was possible to do this without injury as these components are in separate sheaths. Two electrodes *A* and *B* were now applied to the split nerve as shown in Fig. 22 and the reflex threshold (for ipsilateral spinal reflex in tibialis anticus) was determined for each, and was observed for some time until it reached a constant value, which was 60 cm. coil distance

for *A* and 33·9 cm. for *B*. Division *L* was now cut at *X*, care being taken not to disturb the nerve between either pair of electrodes. The threshold

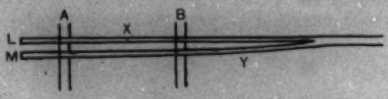

Fig. 22. Diagram, explanation in text.

at *A* was now found to have risen to 58 cm. in the course of about 2 min. The excitation from the cut nerve was probably responsible for this delay. One would infer from this increase of threshold that the lowest threshold fibres were in the cut division *L*. The threshold of *B* was found unaltered. Division *M* was then severed at *Y* with similar precautions and the threshold at *B* was found to increase to 33·2 cm., again in the course of about 2 min. As before one would now infer that the lowest threshold fibres were in division *M*. As it seems unlikely that there is an alteration in the relative excitability of the fibres of *L* and *M* divisions over so short a distance as *AB*, one must accept the alternative that the thresholds measured were not afferent fibre thresholds but neurone thresholds. The lowness of the reflex threshold for the whole nerve must have resulted both at *A* and at *B* from the summation of the results of two component stimuli, each in itself subliminal (as regards reflex effect), the one in the *L* division of the nerve and the other in the *M* division. Such summation must be referable to a subliminal central effect from *L* combining with a subliminal central effect from *M*. Cutting half the nerve proximal to electrode *A* in the first observation and proximal to *B* in the second reduced the number of such excited afferent fibres and so necessitated an increase of stimulus in order to excite fresh afferent fibres to replace those removed by the section. Repetition of this experiment on another preparation gave identical results.

Discussion.

Experiments demonstrating spatial summation in occasional observations have been previously described (3, 5, 9); in the present investigation it has been found in almost every case whether the stimuli were applied to the same nerve or to different nerves. Though no reflex was produced by either stimulus alone, nerve impulses must have travelled up some afferent fibres. This shows that in the spinal or decerebrate flexor reflex a single impulse in a single afferent fibre is rarely, if ever, a

stimulus of more than subliminal value. Facilitation either spatial or temporal is necessary for reflex discharge. The design of the central nervous system is as favourable for spatial summation as the discharge of the receptor organs is for temporal summation. Where is the central locus of this process of summation of the excitatory effects of single impulses in single afferent fibres? Undoubtedly spatial summation must occur at the place of convergence of the afferent fibres on the final common path, *i.e.* at the synapse or in the neurone itself central to the axon hillock, and from its close similarity to spatial summation it is likely that temporal facilitation occurs at an identical place. When the propagated disturbance reaches the region where summation can occur, it is not transmitted straight through, but is changed to an excitatory state which, if of sufficient intensity, sets up a fresh propagated disturbance, the reflex discharge. This requisite degree of excitation for initiating a discharge down the axis cylinder of the neurone can only be attained by the summation of the excitatory states produced by the arrival of several afferent impulses. Weak stimuli give rise to a threshold excitation in very few neurones, but have a considerable subliminal influence—the subliminal fringe. Strengthening of the stimulus adds to the excitation existing in these neurones, raising it to above the threshold value; fresh neurones are, however, added to the subliminal fringe. With strong stimuli practically all the neurones may be brought into the supraliminal group, the subliminal fringe then ceasing to exist (*e.g.* peroneal nerve on spinal semitendinosus). It is the subliminal fringe which makes facilitation possible.

Reduced reflex time is probably mainly occupied in the time taken for excitation to attain neurone threshold. In the flexor reflex the response to a single stimulus is usually single because the rate of disappearance of the central excitatory state is so rapid. The same explanation accounts for the facility with which excitatory rhythms are transmitted as response rhythms. As yet we do not know the effect of a reflex discharge on the excitatory level of the neurone, but it seems likely that there will be exhaustion of some central excitatory state, a condition tending to prevent further discharges. The overlap of excitatory afferents on any particular neurone and the power of summation is such that the accumulation of excitation may be great enough to cause several discharges—after discharge and secondary waves.

At first sight it seems difficult to reconcile our results with experiments(18) showing that, in the tetanic spinal flexor reflex, there is no

increase in the number of excited motor units with continuance of the stimulation (*d'emblée*). These results were obtained with strong stimulation at a comparatively slow rate (20σ between stimuli). With strong stimuli the subliminal fringe is relatively very small in comparison with the above-threshold excitations, hence the difficulty of detecting any minute amount of temporal facilitation which might occur. Reflex facilitation between two successive stimuli to the same afferent was found in isolated experiments by Forbes, Querido, Whitaker and Hurxthal(14), and an optimum interval of 15σ to 30σ was noticed. That they did not find it more often is perhaps to be attributed to their use of stimuli well above threshold. Others(28, 2) have failed to observe it for the same reason.

The significance of the various facilitation curves is by no means clear. Undoubtedly they are related to the values of central excitatory state at the indicated intervals. With tibialis anticus we have been able to explain the curves as complexes of two types of central excitatory state and to show that the delayed component is produced by the tension afferents of gastrocnemius-soleus, being probably of the nature of a rebound following an initial inhibition. Semitendinosus has displayed a certain uniform behaviour with any particular pair of afferents, and doubtless the facilitation curves obtained are compounded of the effects of central excitatory states with characteristic time processes. This complexity of central excitatory state following a single afferent volley was most unexpected. It is a striking illustration of the mixed character of afferent nerves(26, 27) and serves as a warning against too simple a conception of what has hitherto been regarded as an extremely simple type of reflex response—the ipsilateral spinal flexion reflex. Moreover it indicates the disadvantages of nerve stimulation in the study of reflexes, for under these conditions afferent fibres of the most diverse types are stimulated, and the central neurones are subjected to the simultaneous arrival of these propagated impulses—a circumstance far removed from physiological conditions.

Summary.

The central condition produced by a single volley of afferent impulses in an ipsilateral flexor centre has been investigated by sampling with a second afferent volley (usually in another nerve) at various time intervals after the first. Under these conditions, when each reflex stimulus was just threshold, reflex responses were produced at certain stimulus intervals. This can be explained only by the effects of central summation.

Curves can be drawn in which the intervals between the stimuli are plotted against the tensions of the reflex responses.

With the twin nerves of gastrocnemius acting as afferents reflexly on tibialis anticus there is often an optimum degree of facilitation at simultaneity of the stimuli and also with the stimulus to one or the other nerve leading by an interval of about 12σ. This double effect was traced to two separable central excitatory states, one "direct," the other "delayed." Evidence is given that this second effect is evoked by the tension receptors of gastrocnemius, and is possibly a rebound following an initial inhibition. There is thus a complex central condition following a single afferent volley.

When semitendinosus was similarly excited from various nerve pairs complex curves were also obtained, but no attempt at analysis has been possible.

The most striking feature of the results has been the comparatively long duration of the central effect of one threshold stimulus as shown by its power of facilitating the effect of a second threshold stimulus. This shows that the first single afferent volley must produce an enduring central state—the central excitatory state—which is capable of summation. Moreover, since we find facilitation between threshold stimuli is almost universally demonstrable, it would seem that a single afferent impulse is by itself unable to produce a threshold central excitatory state in a neurone. Summation is necessary for every reflex discharge. Reflex threshold or neurone threshold is to be distinguished from afferent nerve threshold.

The enduring central excitatory state is responsible for after-discharge, an extreme example of which is described in a de-afferented tibialis anticus of a chronic spinal dog.

We have to thank the Medical Research Council for defraying in part the expenses of this investigation; and the Royal Society for permission to reproduce Plate-fig. 2 from *Roy. Soc. Proceedings*.

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612.178.019.7 Scyllium

ON THE INFLUENCE OF THE EXTRA-CARDIAC NERVES UPON SINO-AURICULAR CONDUCTION IN THE HEART OF *SCYLLIUM*.

By J. J. IZQUIERDO[1] (Mexico City).

(*From the Laboratory of the Marine Biological Association at Plymouth.*)

In a previous paper (1) I have shown that the favourable action of sympathetic stimulation upon conduction in the auriculo-ventricular bundle and the tissues forming the basal segments of the cold-blooded heart is not seen in the mammalian auricle. Moreover, in accordance with the observations of Lewis and collaborators (2, 3), which I have confirmed, relief of intra-auricular block in the dog's auricle occurs after stimulation of the vagus nerve and not, as in the turtle, after stimulation of the sympathetic nerves (4). Thus, it has already been proved that differences exist between these types of heart and that Gaskell's (5) generalization that "the innervation of the hearts is the same both in cold-blooded and warm-blooded animals" no longer holds.

Results have been obtained which suggest that a division into two groups, namely, the "cold-" and "warm-blooded" hearts, which include a large number of animals presenting differences in organization and functional correlations is premature and that many types must be investigated in detail before any division can be attempted.

The results reported in this paper, in addition to their value from a comparative point of view, are of importance in connection with the problem as to whether cardio-sympathetic nerve-fibres exist in the fishes. It has been repeatedly denied that any accelerator augmentor effects are seen on stimulating these nerves, but their influence upon conduction has not yet been studied. It is conceivable that the influence upon conduction might alone be present; if no such effect could be detected, then the evidence against the existence of cardio-sympathetic nerves in the fishes would be still stronger.

[1] Fellow of the Rockefeller Foundation.

GENERAL CONSIDERATIONS OF METHODS.

The existence of a cardiac branch of the vagus and its distribution in the sinus venosus has been long recognized in the fishes (Müller(6), Stannius(7, 8), Kasem-Beck and Dogiel(9), Chevrel(10)) and the results of stimulating it have been reported. The effects seen are not very prominent in some of the lower fishes (lampreys(11)) but are more constant and definite in the higher types (Cadiat(12), McWilliam(13), Skramlik(14), Blaschko(15)). On the other hand, the existence of sympathetic nerves to the heart has been denied both on anatomical and physiological grounds (see Tigerstedt(16), Brücke(17), Clark(18)).

Stannius and Chevrel, who made an extensive investigation in more than 100 species of fishes, found no evidence of any sympathetic nerves going to the heart[1]. The lateral chains which have been described in lampreys (Johnston(19)), which at first sight could be taken as belonging to the sympathetic system, originate from the vagi according to the embryological studies of Kunz(20). The methods for studying the degeneration of the extra-cardiac nerves or differentiating them intra-cardially by selective strains are immature (see Fukutake(21), Woollard(22), Okamura(23)) and at the moment offer no solution of the problem.

It is true that some anatomical (Gegenbauer(24), Kolf(25)) and experimental evidence has been adduced in favour of their existence (Kasem-Beck and Dogiel(9), Hemmeter(26)), but the statements are vague and being unaccompanied by tracings are of doubtful value. Moreover the accelerator, augmentor or dromotropic vagal after-effects, so prominent in amphibians (Mills Wesley(27), Gaskell(28), Skramlik(29)) and in turtles (Garrey(30, 31)) have never been observed (McWilliam(13), Botazzi(32), Wilson Green(11), McQueen(33), Skramlik(14), Blaschko(15)) in the fishes.

The preparation of the vagi for stimulation does not offer any difficulty, but owing to the meagre information available about the cardiac sympathetic (Botazzi(32), Chevrel(10), Daniel(34)) it was thought desirable to make several careful preliminary dissections. They were made in fresh specimens under a binocular microscope and magnification × 24. Some of them were made on animals previously injected with methylene blue and in others osmic acid 2 p.c. was used at different stages of the dissection.

[1] It is regrettable that, for the purposes of morphological and functional investigation, nobody has been fortunate enough to obtain *Diodon*, the *raro piscis* in which Stannius reported to have found cardiac sympathetic nerves.

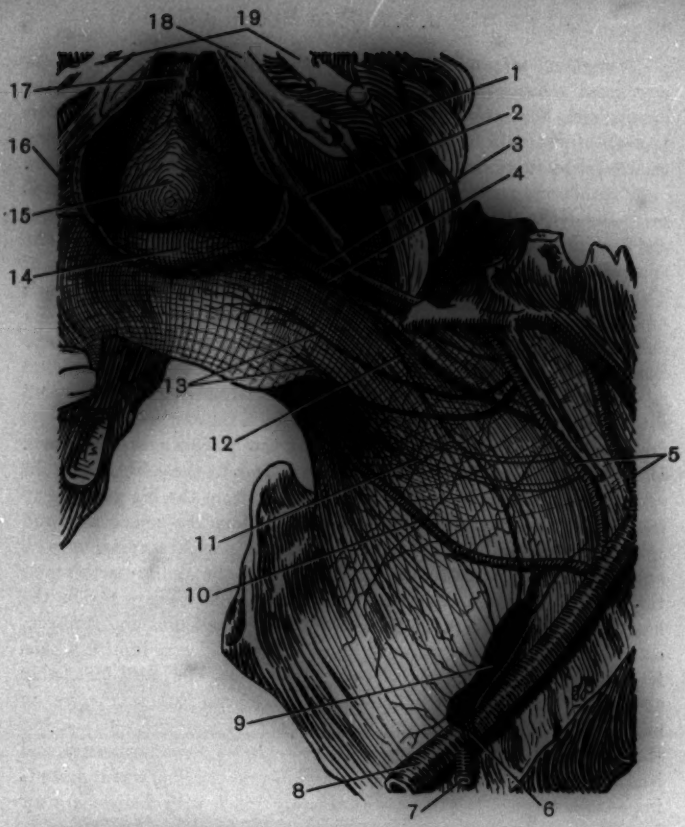

Fig. 1. To illustrate the relation of the cardiac vagus and sympathetic nerves in *Scyllium*. The structures represented in the upper left and lower right corners are situated in the normal animal ventrally and dorsally to the œsophagus and pharynx. The œsophagus has been dissected out. (1) Branchial filaments; (2) remainings of the fifth ceratobranchial cartilage; (3) ductus Cuvieri; (4) cardiac branch of the vagus coursing along the walls of the ductus Cuvieri and the ventral side of the fifth branchial arch; (5) branchial afferent arteries; (6) posterior roots of the elongated dorsal ganglion (9); (7) coeliac axis; (8) dorsal aorta; (10) axillary artery; (11) post-branchial plexus; (12) thick sympathetic branch joining the vagus cardiac branch; (13) vagal œsophageal branches; (14) sinus venosus; (15) auricle; (16) thin pyramidal muscle covering the fifth branchial arch; (17) bulbus aortæ; (18), (19) third and fourth branchial arches.

Though no attempt was made by these means to obtain definite anatomical solution about the existence, number or path followed by the sympathetic fibres, a very good orientation was obtained of their most probable course and of the points which should be selected for placing the electrodes in attempting to get a functional solution of the problem.

If an antero-posterior section of the dorsal body wall of *Scyllium* is made slightly to the left of the median plane (Fig. 1) and the œsophagus carefully dissected out, an idea can be obtained of the relations of the sympathetic with the cardiac vagal branches and with the heart itself. The most striking structure among numerous connective tissue threads uniting the œsophagus and dorsal bundle of muscles is a relatively elongated ganglion already noticed by Giltay(35), Remak(36) and Stannius(7), and better described by Chevrel(10). It is always found in close relation with the first inter-renal body, lying upon a small arteriole branching from the axillary artery. It is, as Béraud(37) described it, suspended in the cavity of the posterior cardinal vein. Usually another smaller ganglion is found near to the middle line. In addition to numerous branches which radiate from the big ganglion to the stomach and œsophagus and posteriorly join the cœliac axis and splanchnics or establish connections with the first five spinal nerves, another group of fibres courses ventrally along the axillary artery and the walls of the posterior cardinal vein, towards the axillary region, the heart, sinus Cuvieri and ventral aspect of the œsophagus and stomach, establishing connections with the vagus[1] and hypoglossus branches, thus forming the post-branchial plexus. It would be desirable for testing the physiological effects of possible cardiac sympathetic nerves to stimulate at different points along the route taken by these nerves, but the dissection required to expose all these structures is extensive and leads inevitably to the opening of very thin-walled veins, which make it impossible to keep the heart moderately filled with blood—a very important condition—so the method cannot be employed.

Direct stimulation of the elongated ganglion, the method used by Bottazzi, is to be ruled out for the same reason. Stimulation of the vagus itself after sectioning all its branches except the cardiac branch, the method which has been used for investigating the absence of vagal after-effects or of augmentor or accelerator effects after atropinization (Skramlik, Blaschko) had given negative results. But from the

[1] Stannius, on p. 132 of his book(7), clearly refers to these connections but did not suspect that they might represent the channels bringing sympathetic fibres into the cardiac branch of the vagus.

dissections made, it appeared that the main sympathetic fibres might well reach the heart along the walls of the ductus Cuvieri[1] and get admixed with those of the vagus after the cardiac branch of this nerve has entered the venous walls at the point where the big anterior and posterior cardinal veins join. It was therefore decided to put and keep fixed in position during the whole experiment the two stimulating electrodes upon the posterior walls of the right and left ducti Cuvieri, and so to study (*a*) the predominating vagal effects and possible sympathetic after-effects in the normal fresh preparation, and (*b*) the existence of pure sympathetic effects after atropinization.

If the heart is reached by the ventro-lateral route, after cutting the choracoid and the group of muscles attached to it (Hoffmann(38), Kolff(25), Hemmeter(26), Skramlik(14), Blaschko(15)) in addition to the inevitable trauma made in its vicinity, the sinus venosus and the ventricle remain more or less hidden from the observer and as Hemmeter pointed out, the cardiac nerve lies deep behind the upper border of the ductus Cuvieri, along the fifth branchial arch.

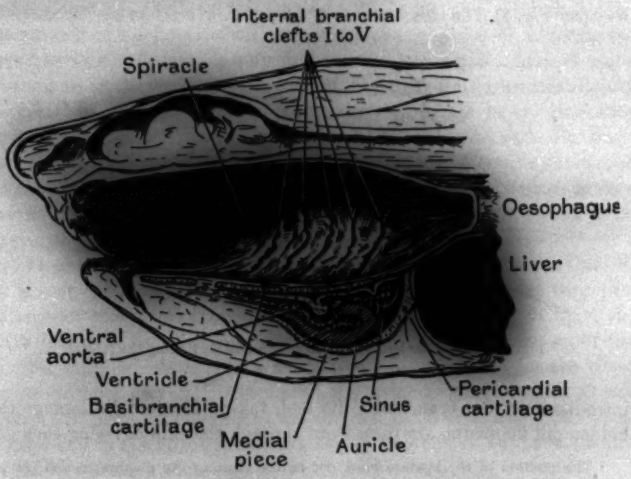

Fig. 2. Semi-schematic median section of *Scyllium* to illustrate the relations of the pericardial chamber to the pharyngeal floor.

[1] It was shown by Kasem-Beck and Dogiel(9) that nerve fibres also enter the fish heart along the coronaries, but no experiment was tried with them.

Now, inspection of a medial section of *Scyllium* (see Fig. 2) shows that the heart segments and nerves entering it are much more easily reached through the pharyngeal cavity from which they are only separated by a thin cartilaginous layer and the mucosa. A method was devised for exposing the heart and ducti Cuvieri of *Scyllium* from this aspect, which requires little dissection in the vicinity of the heart and ducti.

The heart and cardiac-nerve preparation in *Scyllium*.

The *Scyllium* was pithed in the usual way, a wire being passed through its spinal canal and the cranial cavity packed to avoid excessive bleeding. After putting it on its back, the abdominal cavity was amply opened to gain free access to the viscera; the vessels going to the liver and intestinal tube ligatured, these organs dissected off and a ligature passed transversely through the dorsal bundle of muscles and tied around the vessels[1] upon the dorsal wall.

The pharynx was next[2] prepared and its floor exposed in the following way (see Fig. 3). The closed points of a fine pair of scissors are successively

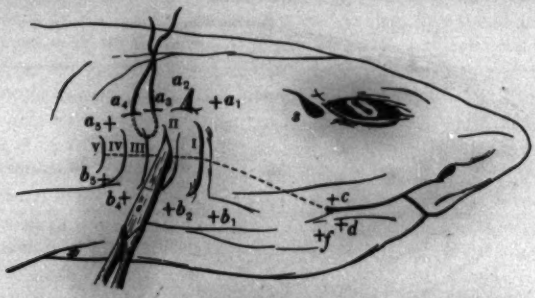

Fig. 3. Method for ligaturing the vessels and sectioning the lateral bucco-pharyngeal wall of *Scyllium*.

introduced by the branchial clefts and, taking care not to damage the hidden gill filaments, are passed out through the skin covering each gill

[1] The position of the ligature must not be too close to the diaphragm and dilated portions of the posterior cardinal veins; otherwise the preparation is damaged and does not remain active for long.

[2] If the branchial vessels are prepared first and then those below the diaphragm tied, the heart is found afterwards extremely distended with blood, which acts unfavourably upon the preparation.

pouch and then separated, to form two rows of holes above (a_1 to a_3) and below (b_1 to b_3). Another similar opening (f) is made by piercing the buccal floor immediately behind the lower jaw's posterior border and two more (c and d) above and below the buccal commissure. A series of strong threads are passed through the following openings: c to spiracle s, spiracle to a_1, d to f and f to b_1, and after all these have been tied firmly the bucco-pharyngeal wall is sectioned, up to the first branchial cleft, as indicated by the dotted line. In the same way threads are passed through the holes in front and behind the upper and lower segments of each branchial arch, and tied; the arches being sectioned in between, one after the other, except the last or fifth. After repeating the same procedure on the other side, lateral sections enable the pharyngeal cavity and its floor to be well displayed without any important loss of blood. Finally, to make the preparation handier, the body is separated by making a section distal to the ligature below the diaphragm. The preparation is then fixed to a small cork-covered board by clamping the two pectoral fins by means of two paper clips and by pinning it down, taking care not to puncture a vessel. By lifting and maintaining the dorsal half of the preparation upright the pharyngeal floor, covering the heart and ducti Cuvieri, is very well exposed (see Fig. 4). After cleaning the mucus on the surface, the mucosa is dissected off from an area slightly larger than the one actually needed to expose the heart, extending tailwards to the fine muscles covering the fifth branchial arch. The heart is exposed by cutting out an area of the thin cartilaginous pericardial chamber: to make a first cut the point of a fine pair of scissors is very carefully introduced through a point K in the edge of the medial basal piece, and the opening gradually enlarged.

To expose the nerves along the posterior walls of the ductus Cuvieri, it is only necessary to tear apart, little by little, the two very thin triangular muscles i, i' (inter-arcuales, see also 15 in Fig. 1) upon the fifth branchial arches. One must be satisfied with an exposure of the ductus wall—which would be difficult to see were it not for the blood in it—without attempting to dissect the very fine nerves which at this point, and from the junction of the anterior and posterior cardinal veins, course first in the thickness and along the inner surface of the wall, to the sinus venosus.

As the trauma near the heart is so slight and as this organ is kept moderately filled with blood, the heart thus prepared beats regularly for a long time, but if these precautions are not taken the heart soon becomes irregular and stops. Nevertheless the reputation that the fish's

heart and especially that of *Scyllium* has gained of not being convenient for carrying out physiological experiments (Mills Wesley(37), Straub(39),

Fig. 4. The heart-nerve preparation of *Scyllium* after the two lateral sections have been completed and the bucco-pharyngeal floor exposed as described in text. Posteriorly to a line between the posterior borders of the two pyramidal muscles covering the fifth branchial cartilages a portion of the mucosa has been dissected out to leave bare these muscles and the cartilaginous pericardial roof. *K* shows the point in the edge of the medial cartilage at which the opening of the pericardial chamber is begun, and *i*, *i'* the position of the electrodes.

Brücke(17), de Boer(40)) is at least partially true, because it must be stated that the statement that the hearts keep beating for many hours applies only to the basal segments. In hearts prepared and kept in the best conditions an A.-V. block always develops a few hours afterwards.

Platinum hook electrodes were carefully placed upon each of the posterior walls of the two ducti Cuvieri (points *i*, *i'* in Fig. 4) and the different heart segments connected by means of very fine serre-fines and

silk threads with very light recording levers. As the preparation was used for several hours (sometimes the effects were tested after 18 hours) a moist chamber was provided by putting pieces of cotton-wool saturated with sea water around the heart without interfering with the thread's movements.

The faradic current from an ordinary induction coil with a 2-volt accumulator in the primary was used for stimulating the nerves at the rate of five double shocks per second and rarely at the frequency of the coil's interrupter.

The movements of the recording levers, as well as those of a magnetic signal included in the primary to register the stimulating shocks, were recorded simultaneously upon bromide paper by placing them in front and casting their shadows upon the slit of a moving-paper camera. Time was signalled by interrupting the beam of light every second.

In addition, in order to impair conduction, the two fine flat-surfaced jaws of a clamp were placed from above and maintained at both sides of the sino-auricular junction, pressure being progressively exerted by means of a fine screw during the experiment.

After observations had been made upon the normal preparation, 0·01 p.c. atropine in sea water was dropped upon the heart and ducti and without altering the connections with the writing levers, the position of the electrodes or any other condition of the experiment, further observations were made upon the effects of nerve stimulation, after the vagus paralysis was completed.

Twenty *Scyllium* (both *catulus* and *cannicula*) were tested.

Results.

A. *Normal hearts.*

When the nerves coursing along the walls of the ducti Cuvieri of *Scyllium* are stimulated in the way which has been described, two groups of effects are seen: (*a*) Coincident with the slowing of the heart rate the conduction intervals between sinus and auricle are increased or block produced between these two heart segments. (*b*) When stronger stimuli are used these primary vagal effects are followed by an extremely brief secondary phase of opposite character.

1. *Conduction between sinus and auricle.*

(*a*) *Primary vagal effects.* When the tissues between these two heart segments are kept under normal conditions no change in the S.-A.

Fig. 5. Primary effects exerted upon the S.-A. intervals, previously lengthened by slight pressure, by two vagal stimulations which slow the heart. The effects are seen to extend over the two or three beats following the end of the stimulation. In this and in other records the measured values for S.-A. intervals are written below the upper curve (A = auricular mechanogram, which is read upwards), and values for sinus-sinus intervals, giving the duration of each cardiac cycle (S = sinusal mechanogram, to be read downwards), are written below the lower curve. At R_1, stimulation of the nerves of the right side with five double faradic shocks per second; coil distance = 6 cm. Time marker in this and other records = 1 second.

Fig. 6. A second tracing taken later from the same preparation when sino-auricular conduction had been more profoundly impaired by increasing the clamp pressure. At R_1, right side nerves stimulated; coil distance = 5 cm.

Fig. 8. Primary effects of a nerve stimulation (at R_1, with coil distance = 4 cm.) which produces a partial (2 : 1) S.-A. block. Sufficient clamp pressure had been exerted to lengthen the S.-A. intervals. The slowing of heart-rate and lengthening of S.-A. intervals are seen to extend to the three beats following the end of the stimulation.

Fig. 9. After a nerve stimulation sufficient to slow the sinus and produce total S.-A. block a slight acceleration and improvement of S.-A. conduction is seen immediately after the end of the stimulation. Effective pressure was exerted between sinus and auricle At R_1, stimulation of the nerves of the right side; coil distance = 4 cm.

intervals accompanies the other effects of vagal stimulation. Only when conduction is impaired by placing a clamp between them the following changes take place.

(α) Stimulation of the vagus nerve which slows the sinus rhythm, lengthens the conduction between sinus and auricle (see tracings in Figs. 5, 6 and the graph in Fig. 7). A slowing of rhythm of the magnitude

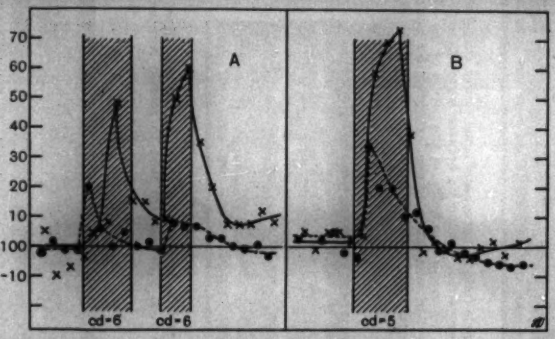

Fig. 7. The variation of the sinus-sinus intervals (dotted line) and the S.-A. intervals (crossed line) of successive beats have been calculated in percentages of the values before stimulation and plotted together. The shaded areas show the period during which the right side nerves were stimulated.

seen in the experiments (reaching from 8 to 34 p.c.) by itself should rather improve conduction. But the opposite effect occurs (increases reaching to 50–75 p.c.). The lengthened intervals persist for a few beats after stimulation is over.

(β) When a stronger stimulus is applied which produces a partial block (see tracings in Fig. 8), the S.-A. intervals in the beats passing through are lengthened and gradually return to normality after stimulation is over.

(γ) With an even stronger stimulus a greater slowing of the fundamental cardiac rhythm occurs accompanied by the production of a total block (see tracings in Fig. 9). After the end of the stimulation the complete block disappears and in the beats immediately following the S.-A. intervals are shortened. They will be considered in detail immediately.

(δ) Intense stimuli, especially if applied upon the nerves of the left

Fig. 10. Simultaneous records of the ventricle (upper curve), auricle (middle curve) and sinus (lower curve) of a normal heart of *Scyllium*. Ventricle and auricle are read upwards; sinus downwards. Conduction between segments kept normal (no pressure applied). Two strong stimulations of the same intensity (coil distance = 4 cm.), first of the nerves of the left side (L_4) and then of those of the right (R_4), stop the whole heart. An after-effect of stimulation is seen extending for approximately ten beats in both instances, in which the intervals are definitely shortened. Slight accelerator effects or no shortening of the A.-V. intervals accompany this after-effect.

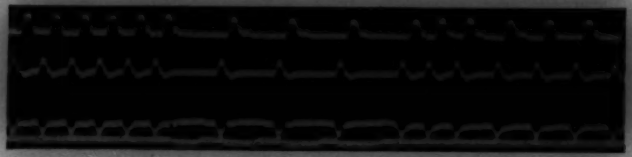

Fig. 12. During a remarkable slowing of the rate of a normal heart of *Scyllium*, produced by properly timed stimulations of the nerves of the left side (at L_3; coil distance = 3 cm.), the S.-A. intervals are seen to remain constant. (A = sinusal and V = ventricular mechanograms, to be read upwards.)

Fig. 13. Effects exerted by a stimulation of the nerves of the left side (at L_3; coil distance = 2 cm.) upon the *atropinized* heart of *Scyllium*. The shortening of the S.-A. intervals still persisted by the twenty-fifth beat following the end of the stimulation. (See also Fig. 14.)

side, stop the whole heart[1] (see tracings in Fig. 10) and the after-effect already mentioned in the last paragraph is again seen.

(*b*) *The after-effects of vagal stimulation.* We have already seen that in cases (α) and (β) the S.-A. intervals are found still prolonged in the beats immediately following the end of the stimulation, and gradually return to their initial values. More rarely, especially when the stimulation is applied when the intervals are still sub-normal as a result of the after-effect mentioned, the lengthening can be observed extending over a long series of beats. But in the two last cases (γ) and (δ) (*i.e.* with the stronger stimuli) the S.-A. intervals of the beats immediately following the stimulation are found to be shortened. In Fig. 11 the data partly repro-

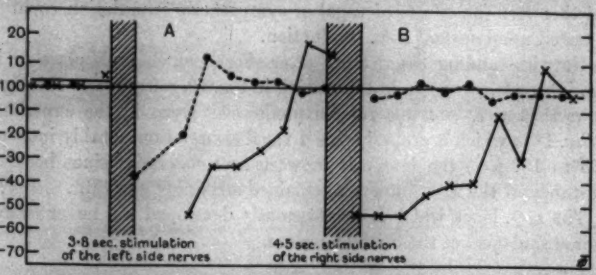

Fig. 11. Variations of the sinus-sinus intervals (dotted line) and S.-A. intervals (crossed line) in the beats following the complete arrest of the heart (Fig. 10). Both are expressed in percentages of their initial values. It is shown that the shortening of S.-A. intervals is independent of any change of rhythm and that it is not counterbalanced when an accelerator after-effect has developed at the same time.

duced in tracings in Fig. 10 have been plotted in percentages to illustrate the point. The S.-A. intervals in the first seven to ten beats were notably reduced in length, and the shortening reached as much as 81 p.c. after the first stimulation was over, 50 p.c. after the second. No change of rate occurred after *B*, but after *A* a notable acceleration of about 40 p.c. was observed, extending to only three or four beats (extremely brief accelerator after-effects). It is interesting to add that for this effect no

[1] On account of the difficulties in writing the movements of the sinus upon smoked surfaces, the assumption that the vagus not only stops the whole heart by production of an S.-A. block but also stops the sinus, has been supported mainly by direct observations made under a magnifying glass (Skramlik, Blaschko). As far as I am aware, no tracings giving the graduated effects upon the sinus have appeared before in the literature, for fishes or other cold-blooded hearts.

clamping of the S.-A. junction is required. Experiments in which S.-A. conduction is impaired nevertheless show the same after-effect.

As in some of the tracings the shortening of the S.-A. intervals is observed while the sinus is beating slower than before stimulation (tracings in Fig. 11) it could be argued that the former effect is purely the result of the slowed rate of beating, but as Fig. 11 *B* shows, the same effect also develops when no change of rhythm occurs or even when the rate of beating is increased, which by itself should bring about the opposite effect[1] (as in *A* of the same figure).

2. *Conduction between auricle and ventricle.*

Attempts to put a clamp upon the A.-V. junction were unsuccessful and therefore the effects of vagal or sympathetic stimulation could not be tested upon normal A.-V. conduction.

Notwithstanding the definite after-effects which took place in the S.-A. intervals, as shown in the tracing in Fig. 10, reference to this figure shows that A.-V. intervals remain unaltered. Even in the experiment in Fig. 12 in which a properly timed vagal stimulation notably increased (105 to 138 p.c.) the intervals between four successive sinus beats the constancy of the A.-V. intervals remained strikingly constant.

The A.-V. block which is spontaneously developed was never relieved after stimulation of the ductean nerves.

B. *Effects after atropinization.*

Several minutes after the heart and ducti Cuvieri were bathed with 0·01 p.c. atropine, which was without any effect upon the rate of beating, and without changing the position of the electrodes or the writing levers, new stimulations were made of different durations, with different coil distances and with different periods of interruptions (from five double shocks previously used to the frequency of the coil's vibrator). The alterations in the rates of conduction associated with the vagal stimulation were no longer observed. Instead, the opposite effects of quickening of rhythm and shortening of S.-A. intervals were observed on a few occasions (see accompanying table).

The results of one of these experiments (in the same preparation which had given tracings 5–9 and 10 before atropinization) are given in the tracing in Fig. 13 and in the graph in Fig. 14 in which the corresponding variations have been plotted in percentages of the initial values. The

[1] Hoffmann(41) considered that in *Esculenta* the effect produced upon conduction by the pure slowing of rhythm is still bigger than the one exerted by the vagus.

TABLE I. Effects of stimulating the extra-cardiac nerves of atropinized *Scyllium* upon Sinus-Sinus and S.-A. intervals.

Number of *Scyllium* and record		Nerves stimulated, duration of the stimulation and coil distance	Sinus-Sinus intervals		S.-A. intervals	
			In seconds	Change p.c.	In seconds	Change p.c.
11, D	Before stimulation (9)*		3·24	—	0·35	—
	During stimulation (2)	Right side; 6·4 seconds; *cd* = 2	3·07	− 5·2	0·34	− 2·9
	After stimulation (6)		3·03	− 6·5	0·33	− 5·7
	(6)		2·98	− 8·0	0·37	5·7
13, E	Before stimulation (4)		2·26	—	0·60	—
	During stimulation (4)	Left side; 9·1 seconds; *cd* = 0	2·27	—	0·49	− 18·4
	After stimulation (6)		2·01	− 11·1	0·48	− 20·0
	(6)		2·05	− 9·3	0·48	− 20·0
17, H†	Before stimulation (7)		2·06	—	1·10	—
	During stimulation (5)	Left side; 9·5 seconds; *cd* = 2	2·02	− 2·0	1·05	− 4·5
	After stimulation (6)		2·00	− 3·0	1·06	− 3·6
	(6)		2·00	− 3·0	1·04	− 5·5
	(7)		2·01	− 2·5	1·09	Back to initial value
17, I†	Before stimulation (7)		1·98	—	1·10	—
	During stimulation (7)	Left side; 14·5 seconds; *cd* = 2	1·96	− 2·0	1·02	− 7·3
	After stimulation (6)		1·91	− 3·5	0·93	− 15·5
	(6)		1·91	− 3·5	0·93	− 15·5
	(6)		1·96	− 2·0	0·97	− 11·8
	(5)		1·92	− 3·0	0·96	− 12·7

* The figures between brackets indicate the number of beats from which the averages given were taken. Read downwards, those corresponding to periods after the stimulation indicate successive series of beats.

† In these two observations pressure was applied upon the S.-A. junction.

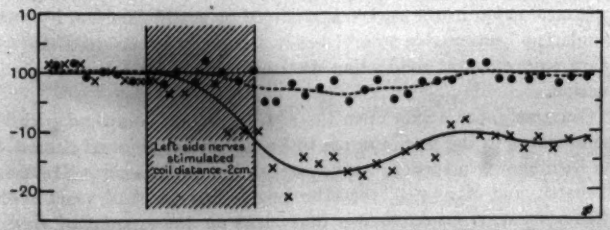

Fig. 14. The values for the sinus-sinus intervals (dots) and for the S.-A. intervals (crosses) in Fig. 13 have been calculated in percentages of the values before stimulation and plotted together. Very slight change of the cardiac cycles follows the stimulation, but the S.-A. intervals are definitely shortened.

clamp had been re-applied to greatly impair S.-A. conduction (1·1 seconds average initial value) and very strong stimulus at the rate of five double shocks per second was applied for the long period of 15 seconds. Very slight changes in rate (no greater than 5 p.c.) followed the stimulation, but the S.-A. intervals are definitely shortened over 15 p.c. immediately after stimulation and return so gradually towards the initial values that these have not yet been reached at the twenty-fifth beat.

Discussion.

From the nature of the other effects accompanying the changes in conduction observed after stimulation of the nerves coursing along the ducti Cuvieri of *Scyllium*, it has been easy to recognize that under these circumstances the effects of vagal stimulation predominate. During these periods S.-A. blocks are produced, and the corresponding conduction intervals are lengthened when conduction between the two heart segments has been impaired. But A.-V. conduction is not modified.

These results fall in line with the results of stimulation of the vagus nerves upon conduction reported in other cold-blooded hearts. But quantitative differences undoubtedly have to be recognized as to the range to which the slowing and lengthening actions spread upon the hearts and in relation to the intensity of the action exerted. The production of S.-A. auricular blocks takes place also in *R. temporaria* (Muskens(42)), crocodiles, turtles and snakes (Gaskell(5)), and bony fishes (Blaschko(15)), but in these, as well as in turtles (Garrey(30)), in *R. temporaria* (Dale and Mines(43), Mines(44)) and *R. esculenta* (Hoffmann(41)), the action seems to spread not only to the auricle but also to the A.-V. junction.

If conduction in the auricle is modified or not in *Scyllium* by nerve stimulation I am unable to say, but it can safely be assumed that the action seen on S.-A. conduction does not extend as far as the A.-V. junction.

Conversely, the action upon the S.-A. tissues, as compared with the one observed in the frog, appears to be of a more profound degree. It has been shown in turtles (Garrey(30)) and in *R. temporaria* (Dale and Mines(43), and Mines(44)) that the lengthening effect of vagal stimulation upon A.-V. conduction is dependent on the amount of slowing produced, *i.e.* that it only results when the rhythm is kept constant but is easily counterbalanced by an opposite change if the slowing of rhythm predominates. In *Scyllium*, according to the evidence presented in this

paper, the lengthening of S.-A. intervals takes place even when very notable slowing of rhythm has been produced.

The changes of opposite character deserve special consideration. In the first place, the shortening of S.-A. intervals is observed only for about ten beats following the stoppage produced by a strong stimulation of the vagus. Secondly, the slight but more prolonged shortening of the S.-A. intervals is observed after intense and prolonged stimulations made in the same preparation after atropinization.

Under similar circumstances very clear augmentor after-effects are also frequently observed. They are rudimentary in magnitude (12–16 p.c. increases) and duration as compared with those observed in higher vertebrates, but it is interesting to notice that, like the effect upon S.-A. conduction reported above, they also extend approximately over ten beats.

If, in addition, we keep in mind that the three or four cycles following a complete stoppage of the heart are constantly found to be reduced in length, it can be said that improvement of conduction seen both in the after-effects and during stimulation after atropinization is accompanied by accelerator and augmentor effects. The results conform qualitatively therefore to those seen in the higher vertebrates, but quantitatively they suggest that in *Scyllium* the sympathetic influence is of a much more rudimentary nature[1]. So appears to be also the influence upon conduction reported in this paper, when compared with the vagal after-effects (Garrey (30)) and the effects of direct sympathetic stimulation (Gault (45), Izquierdo (4)).

Finally, if the two sets of vagal and sympathetic effects are put in front of each other, it is easily seen how they harmonize with the assumption made on anatomical and embryological grounds that in "lower vertebrates the vagi are comparatively large, while the sympathetic trunks are but feebly developed" (Kunz (20)) in the fishes.

Summary and conclusions.

Reasons are given why, to study the influence of the extracardiac nerves of *Scyllium* upon heart conduction, two points in the posterior walls of the ducti Cuvieri were selected for stimulation.

[1] Dreyerre has observed that after adding atropine 1/200,000,000 stimulation of the left vagus at regular periods increased the beats of two *Scyllium* hearts perfused *in situ*. These results were neither reproducible in the same fishes nor obtained in four others in which the experiment was tried. (Personal communication.)

1. In *normal hearts*, stimulation of the nerves coursing along the walls of the ducti Cuvieri bring about two groups of effects upon conduction between sinus and auricle.

(*a*) *Primary vagal effects.* When conduction is impaired by pressure, after a stimulation which only slows the sinus rhythm or produces in addition a partial block, the S.-A. intervals are considerably lengthened, and gradually return to normal after the stimulation is over. The lengthening is independent of the slowing produced.

(*b*) *After-effects.* With stronger stimuli, which definitely slow the fundamental cardiac rhythm and produce total block or arrest the whole heart, these primary vagal effects are followed by an extremely brief secondary phase of opposite character: *i.e.* during the seven to ten beats immediately following the stimulation the S.-A. intervals are found to be shortened, whether conduction has been or has not been impaired by pressure.

Neither normal conduction intervals between auricle and ventricle, nor the A.-V. blocks which spontaneously develop, are influenced by stimulation.

2. *After atropinization* the primary vagal effects are no longer observed. Instead, with strong and prolonged stimuli, the S.-A. intervals are found appreciably shortened both during and after the end of the stimulation. These effects occasionally coincide with accelerator or augmentor effects which, judged by their magnitude and duration, are inferior to those observed in higher vertebrates. These effects qualitatively conform to those seen in the higher vertebrates, but as they are so much less prominent it is suggested that in *Scyllium* the sympathetic action is of a rudimentary nature.

The influence exerted upon conduction in *Scyllium* by the vagus and sympathetic nerves is of the same character as that seen in other cold-blooded hearts. Nevertheless the influence of the vagus upon the S.-A. junction appears to be more profound (not counterbalanced by the slowing of rhythm) than in reptiles and amphibians, nor does it extend to the A.-V. junction. The sympathetic influence is less notable than in the above animals, and similarly does not extend up to the A.-V. junction.

I am much indebted to the Staff and especially to Dr E. J. Allen, Director of the Laboratory of the Marine Biological Association at Plymouth, for their interest and assistance in carrying out this work. My thanks are also due to Dr A. N. Drury for the loan of his moving-paper camera and for his criticism and help in preparing this paper.

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THE SMALL INTESTINE AND GASTRIC SECRETION.

(With special reference to Brunner's Glands.)

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Evidence has repeatedly been advanced that the activity of extracts of the pyloric mucous membrane in causing gastric secretion is also exhibited by those of the small intestine. The present investigation was directed towards elucidating the presence of any structure which might be indicated as concerned in the intestinal phase.

Edkins and Tweedy(1) definitely stated that the duodenal mucous membrane also, though to a lesser degree, acted as the pyloric mucous membrane does. Keeton and Koch(2) believe that a specific hormone is produced in the upper small intestine as well as in the stomach. Babkin(3) in his book points out that although the pylorus and the part of the duodenum in which Brunner's glands are found belong anatomically to different parts of the alimentary canal, they are related functionally, and suggests that this is by reason, possibly, of the similarity of their secretions.

Histologically the pyloric glands and the glands of Brunner in the duodenum show close resemblances. This might suggest that they have some function in common. Pavlov recognized(4) that gastric secretion could be excited from the intestine. Lim(5) set out to investigate the distribution of "gastrin" in the stomach and duodenum with a view to localizing the cells responsible for its production. He found that extracts both of the pyloric region and of the duodenum were effective in producing gastric secretion when injected into the blood; other parts of the small intestine were practically without effect. In his conclusions he considered it not unlikely that the common source of the excitant was the mucoid type of cell which occurs in both regions. Later Ivy, Lim, and McCarthy(6) stated that "an intestinal phase is proven."

Ivy(7) and his co-workers seem to believe, at the present time, in the presence of a gastric stimulant in the blood, and that this can be produced by contact of HCl with the duodenum, and that in duodenal

washings "gastrin" exists. Walawski(8) found duodenal extracts more effective in producing gastric secretion when injected subcutaneously than those of jejunum or ileum.

The object of the present work, then, was to investigate the "intestinal phase" of gastric secretion, and more particularly to determine whether the active part of the duodenum is restricted to that in which Brunner's glands are found, by comparing the response of the fundus of the stomach to intravenous injection of extracts of duodenum in which Brunner's glands were present with that obtained when they were absent.

Method of preparation of extracts.

Extracts of different regions of the duodenum were made by the method of Dalmau(9). Small portions of the mucous membrane and the submucous coat were ground with sand and ten times their weight of *N*/10 HCl. The extract was then boiled for 3 minutes, cooled, nearly neutralized by cautious addition of *N*/2 NaOH, then filtered. The filtrate was added to nine volumes of acetone. The precipitate so produced was filtered off, washed with ether, and dried. The powder was ground with 15 c.c. Ringer's solution, filtered again, and the filtrate used for intravenous injection.

In the first two experiments pig's duodenum was used. Pieces were taken for extraction and for histological examination, so that the results might be correlated. In the pig Brunner's glands go surprisingly far down the duodenum (about 20 cm.). Later, cat's duodenum was used. In the cat there is only a very small region of duodenum containing Brunner's glands. 2 cm. from the pylorus is the absolute limit of these glands. This gave only about 3 g. of tissue for extraction, so that only small quantities of the solutions were available for injection.

Other parts of the duodenum were taken for comparison. The extracts in any one experiment were made from equal weights of the mucous membrane employed. The extracts were numbered in order from the pylorus downward. The injections were intravenous. It has been shown by Gutowski(10) that histamine given intravenously in cats causes practically no response; I have been able to confirm this, so the histamine effect if present at all must have been small.

Method of experiment.

All the experiments were done on cats, and were of the acute type. The animals were fed on milk for the last few days before the experiment.

They were anæsthetized with ether, placed in a warm saline bath, decerebrated, and then allowed a period of half an hour to get over the main effect of the anæsthetic. The œsophagus was tied so as to occlude it and crush the vagi, and a tube was tied into the pylorus. The stomach was washed out till the contents were clean and acid free. In all the experiments recorded even the first washings were clean.

Into the stomach there were introduced 25 c.c. saline; such a volume as could not cause secretion by distension. Then at intervals of 10 minutes three injections, each of 2 c.c. of one of the extracts were made into the external jugular vein. After half an hour (from the last injection) the liquid from the stomach was withdrawn, the stomach washed out and allowed a period of rest before repeating this procedure with another extract for comparison. The potency of the extract was determined by testing the liquid from the stomach with Gunsberg's reagent and then estimating the HCl in a known volume of the liquid after boiling for 3 minutes, by titration with *N*/50 NaOH, using methyl orange as indicator. The pepsin in the liquid from the stomach was estimated by Fuld's edestin method and the results are given in Fuld's units.

The order in which the extracts were used was varied in different experiments so as to exclude the factor of fatigue and delayed effect.

A typical series of experiments is given in the table on p. 51.

A consideration of these figures shows that more HCl and more pepsin were secreted when the extracts injected were made from parts in which Brunner's glands were found to be present than when made from parts in which they were not. Ten experiments are cited and this result is unambiguous in all but one (No. 12). In this one experiment an extract from a part containing no Brunner's glands evoked a secretion containing definitely more pepsin, though also definitely less acid. In three (Nos. 14, 16 and 18) the differences in the amount of HCl secreted were small, but in them the differences in pepsin were clear enough in support of the general inference drawn from the whole series.

In most of the experiments the actual secretions appear to be small. This is no doubt due to several factors; cats do not give, under these conditions, large secretions; and again, it must be remembered that the extent of Brunner's glands, and hence the weight of tissue used in the extractions, was small.

Of course it must be realized that the tissues extracted were unavoidably heterogeneous, but it would seem reasonable to assume that whatever secretagogues or vaso-dilators were present in one part of the duodenum would be present in another close to it, so that in the parts

Exp.	Nature of extract				Brunner's glands, determined histologically	HCl in mg. secreted	Pepsin by Fuld
10	Extract by acetone method from pig's duodenum. Numbers in order from pylorus			(3)	+ + +	21	4
				(2)	+ + +	20	8
				(4)	+	5	2
11	"	"	pig's	(2)	+ + +	5	16
				(3)	0	4	8
				(1)	+ + +	8	48
*12	"	"	cat's	(1)	+	21	16
				(2)	0	13	32
13	"	"	"	(2)	0	0	0
				(1)	+ +	23	8
14	"	"	"	(2)	0	7	4
				(1)	+ +	9	16
15	"	"	"	(1)	+ +	58	32
				(2)	0	0	4
16	"	"	"	(2)	0	14	8
				(1)	+ +	15	32
17	"	"	"	(1)	+ +	44	32
				(2)	0	15	8
				(1)	+ +	45	32
18	"	"	"	(1)	+ +	4	16
				(2)	0	2	8
19	"	"	"	(1)	+ +	19	48
				(2)	0	0	6
				(1)	+ +	13	48
				pylorie		12	64

* The cat from which the extracts were made had previously been used for one of the experiments.

selected for extraction differences of activity if any would depend upon the presence or absence of Brunner's glands. The contrast therefore in the results obtained with extracts of the two different parts must appear to be due to something specific in Brunner's glands capable of causing an undelayed excitation of gastric secretion on intravenous injection. When Brunner's glands were not present in the intestinal mucous membrane extracted very little secretion was obtained.

Summary.

1. The response of the gastric mucosa to intravenous injections of extracts of different parts of the duodenum has been investigated.

2. The results show that when that part of the duodenum which contains Brunner's glands is used, the secretion by the stomach is greater, more HCl and pepsin being produced. When parts that do not contain Brunner's glands are used the effect is insignificant in almost every case.

I have to record my thanks to Dr Nora Edkins for making the histological observations, and to Prof. Edkins for suggestions.

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THE RELATION BETWEEN THE ROD AND CONE MECHANISMS IN THE AFTER-EFFECT OF SEEN MOVEMENT.

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I. Introductory.

It has often been argued that the rod mechanism of the retina represents a more primitive level of development than the cone mechanism. Parsons(1), for instance, considers that the relation between the cone mechanism and the rod mechanism resembles that between the epicritic and protopathic systems of cutaneous sensitivity postulated by Head. On that view the activity of the cone mechanism might inhibit the rod mechanism, as the epicritic system is supposed to inhibit the protopathic system. Experimental evidence of an inhibitory relationship between the rods and cones has been brought forward by Müller(2) in regard to certain phenomena of colour vision, by Creed and Granit(3) in the case of negative after-images, and by Granit(4) in the case of the after-effect of seen movement. On the other hand the results of the experiments of Porter(5) and Ives(6) on flicker, and those of König(7) on the variation of acuity with intensity of illumination, are difficult to explain on the assumption of inhibition; and, in fact, the form of the curves obtained by them suggests that in these cases the rod and cone mechanisms reinforce each other.

The evidence given by Granit in favour of inhibition between the rod and cone mechanisms in the after-effect of seen movement depends on measurements of the duration of the after-effect when a black and white moving object is viewed from various distances at various intensities of the illumination. Granit found that under certain conditions in which both rods and cones tend to be excited the duration of the after-effect is much smaller than would be expected. He attributes this to inhibition of one mechanism by the other. But it may be objected to the results obtained by Granit, that the effects attributed by him to inhibition might be ascribed at least in part to a qualitative change in the

perceptual configuration resulting from the variation in distance of the object from the observer. The present paper describes experiments which were carried out to test Granit's conclusion by a different method.

II. Principle of the method.

It is known that red light of wave length greater than about 6700 Å.U. stimulates only the cones of the retina, and that blue or violet light stimulates the rods relatively strongly and the cones relatively weakly, while light of intermediate wave lengths stimulates both mechanisms to various extents. We should therefore expect on Granit's theory that the duration of the after-effect would depend on the colour as well as on the intensity of the light used to illuminate the moving object. The following method has therefore been adopted:

The after-effect is measured for two different areas of the retina; the light from the moving object stimulating firstly only the rod-free area, and secondly stimulating a larger area. In each case the after-effect is measured for red light of wave length greater than 6700 Å.U. and also for light of at least one other colour, the intensities of the light being approximately equal. Then it is clear that on Granit's theory the ratio of the duration of the after-effect with the larger area to the duration with the smaller area should be greater for the red light than for light of other colours, since when the larger area is stimulated by light of other colours there is mutual inhibition of the rods and cones. That is, if we call the durations of the after-effect for the red light and the large and small retinal areas *LR* and *SR* respectively, and those for the light of the other colour *LO* and *SO* respectively; then on Granit's theory the ratio $\frac{LR}{SR} \Big/ \frac{LO}{SO}$ should be greater than unity.

III. Preliminary experiments with white light.

In some preliminary experiments the apparatus was rather similar to that used by Granit. The observer viewed a rotating drum 6 inches in diameter, marked with black and white stripes half-an-inch wide, from a distance of 7 feet. The retinal area stimulated could be varied by changing the size of a window in front of the drum. The drum was illuminated by a projection lantern in such a way that the illumination of the part visible through the window was approximately uniform, and the intensity of illumination was varied by means of diaphragms of varying aperture. The rest of the room was kept in darkness. In each experiment the drum was rotated for 30 seconds, while the observer

fixated a small point of light in the middle of the visible part of the drum. The drum was then stopped, and the observer recorded the length of time for which it appeared to move backwards.

Measurements were made for two sizes of window (corresponding to visual angles of about 2° and about 5°) and for various intensities of illumination. The results were in general agreement with those of Granit, but it was found that there were very great individual differences between the results obtained by different observers, and between the results obtained by the same observer on different occasions. These differences were considerably greater than those to be expected from the curves published by Granit. For instance, in twenty observations under identical conditions the mean durations of the after-effect for six observers were 8·1, 6·9, 4·8, 0·8, 0·0, and 0·0 seconds respectively. (The last two observers could see no after-effect at all under these conditions.) These individual differences have not been explained.

An attempt was made to measure the after-effect with monochromatic illumination, light filters being placed in the plate-holder of the lantern, but with the available source of light it was not found possible to obtain sufficiently intense monochromatic illumination of the drum by this method.

IV. Experiments with monochromatic illumination.

In a second series of experiments the moving object was in the form of a grating through which monochromatic light was transmitted, and it was viewed through a system of lenses. This arrangement made it possible to use much brighter monochromatic illuminations than in the previous experiments.

Fig. 1 is a diagram of the apparatus. The moving object consisted of a trolley *TT* on which was mounted a system of vertical strings *SS*. These strings were wound over hair combs on a wooden frame. The trolley could be made to move at a slow and uniform speed in the direction of the arrow. Behind the trolley was a box containing a powerful electric lamp L_1, the bulb being of white glass. Light filters could be placed at *FF*. In front of the trolley was a square glass window *WW*, the size of which could be varied, and in the middle of the window was a black spot of 1 mm. diameter which served as a fixation point.

The observer viewed this system through an eyepiece *E* and an object glass *O*. A mirror *M*, from the middle of which a rectangular area of silvering had been removed, a lamp L_2 and filters and dimming slides at *F'F'* made it possible to vary the colour and brightness of an area of

the visual field surrounding the moving object. In all the experiments this surrounding area was of the same colour and intensity as the light

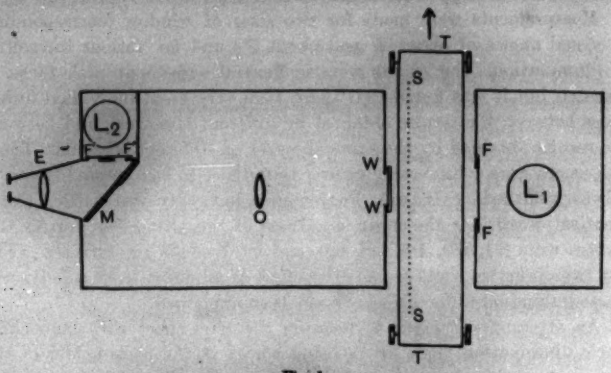

Fig. 1.

Fig. 2.

transmitted through the moving object. The field of view of the observer was therefore as is shown in Fig. 2; the moving lines in the centre of the field being surrounded by a black margin, outside which was a surrounding

area of the same colour and intensity as the bright lines. (It was found that the presence of this illuminated surrounding area made the duration of the after-effect much longer and easier to measure.)

Three different wave lengths of approximately monochromatic light were obtained by the use of appropriate Wratten light filters or combinations of filters—red light of above 6800 Å.U., green light from about 5100 Å.U. to about 5700 Å.U., and violet light from about 3500 Å.U. to about 4600 Å.U. In each observation the trolley was made to move for 40 seconds and then stopped. The observer then continued to fixate the black mark in the middle of the window, and recorded the length of time for which the lines appeared to move backwards. In each series of readings two observations were taken in succession with a given colour and size of window, and the conditions were then changed. The various colours and sizes of window were given in different orders in different series of readings.

V. Results with monochromatic illumination.

A number of experiments were made with windows corresponding to visual angles of 2° (small window) and 4·5° (large window). The intensity of the monochromatic lights was such that they were judged (by direct comparison) to be of the same brightness as a white surface illuminated with an intensity of 2 foot-candles. Table I shows the mean duration of the after-effect for four subjects under these conditions, each of the numbers given representing the mean of twenty observations.

Table I.

Duration of after-effect in seconds

	Red		Green		Violet	
Subject	Small window	Large window	Small window	Large window	Small window	Large window
F. C. B.	12·3	12·4	11·2	13·8	10·4	11·7
J. M. B.	5·7	8·9	7·4	11·6	10·2	10·4
M. E. M.	4·1	5·7	4·0	5·0	3·2	4·8
M. D. V.	5·9	9·1	8·5	8·8	4·4	7·3
Mean duration	7·0	9·0	7·7	9·8	7·0	8·5

The mean value of the ratio $\frac{LR}{SR}/\frac{LC}{SC}$ for the red and green lights is 1·04, and that for the red and violet lights is 1·06. It is quite clear from the variations in Table I that the differences between these ratios and unity cannot be regarded as significant, so that the results under these conditions do not give any evidence of interaction between the rods and cones.

Another series of experiments was made under conditions calculated to increase the amount of rod activity. The large window was made larger so that it now corresponded to a visual angle of 8° (the small window still being 2°), and the intensity of the light was reduced to about 0·05 foot-candles. The subjects were given 1 minute for partial dark adaptation before each observation. Three subjects were employed and twenty observations were made by each subject with each size of window with red and violet lights. The mean values of the ratio $\frac{LR}{SR}/\frac{LC}{SC}$ for the three subjects were 0·72, 0·90, and 0·96 respectively. The fact that these ratios are less than unity might be taken to indicate that the action of the rods reinforces that of the cones under these conditions; but, as in the previous experiments, the differences cannot be regarded as significant.

The subject's introspections did not show any significant qualitative differences in the nature of the after-effect with the lights of different colours. With all colours the after-effect was very variable in its nature. Sometimes it was rapid and sometimes very slow, and it varied from a steady movement coming to a sudden stop to a succession of jerks.

VI. Discussion.

It is difficult to calculate the value of the ratio $\frac{LR}{SR}/\frac{LC}{SC}$ to be expected under these conditions if Granit's theory is true. Granit expresses the intensity of illumination in his experiments as a fraction of "daylight," and not in absolute units; and in several other respects the conditions of these experiments were not strictly comparable with his. But if his explanation be correct one would expect from the curves published by him that the value for the ratio $\frac{LR}{SR}/\frac{LC}{SC}$ in my experiments would be very considerably greater than unity. In the second series of experiments (when the large window corresponded to a visual angle of 8°) one would expect the ratio to be at least 2. It seems, therefore, that Granit's results must be explained by some hypothesis other than that of inhibition between the rods and cones. Perhaps, as has already been suggested, the explanation is to be found partly in the changes in the perceptual configuration when the distance of the moving object from the observer is changed.

It is possible that under conditions in which there is a greater amount of rod activity it may be found that there is some kind of inter-action between the rods and cones in the production of the after-effect. It is

even possible that both in Granit's experiments and in mine the amount of rod action may have been negligibly small. All that my experiments can claim to show is that at present no satisfactory evidence exists of inter-action between the rod and cone mechanism in the production of the after-effect. The evidence of inter-action between the two mechanisms in the production of other visual phenomena seems to indicate the occurrence of inhibition in some cases and of reinforcement in others, as has been pointed out in § I. Much further work will be required before any general statement can be made about the relationship between the two mechanisms.

VII. Summary.

Some experiments have been performed to test Granit's theory that in the production of the after-effect of seen movement the rod mechanism of the retina tends to inhibit the cone mechanism. No evidence of inhibition was found, and it is suggested that Granit's results must be explained in some other way.

I wish to thank Mr F. C. Bartlett, who suggested this research, for his help and advice.

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THE EFFECT OF TEMPERATURE UPON THE ABSOLUTE REFRACTORY PERIOD IN NERVE.

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In the course of a study of the electric response of nerve to two stimuli, Downing and I(1) found that the duration of the absolute refractory period in nerve can be determined with good accuracy by the observation of the stimulus interval at which an increase occurs in the maximal deflection of a sensitive Downing moving-magnet galvanometer, over and above that previously given when the two stimuli were sent in at shorter intervals. The appearance of a second action potential wave is indicated by this increase in electric response, which comes in suddenly as the stimulus interval is gradually increased. The technique used for this determination is fully described in our paper.

As an extension of this study I became interested in the effect of temperature upon the duration of the absolute refractory period, concerning which relationship the literature yielded rather meagre and incomplete information. I therefore carried out a group of determinations on the sciatic nerves of large *R. esculenta*, between 9° and 27° C., with results which were so regular and consistent that it seems wise to put them on record. The data here submitted are in good agreement with values published by a number of other workers, where comparison is possible; their chief advantage lies in the fact that they cover a wider temperature range than has previously been studied by any one investigator, and are more consistent than any to be found in the older literature.

Previous measurements of the duration of the absolute refractory period in nerve have followed either the method of Helmholtz(2), in which determination is made of the least interval between two stimuli applied to the nerve which just produces a summated contraction in muscle, or the method of Gotch and Burch(3) which measures the least interval required for the appearance of a second electrical response in

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nerve. The technique used in the present work is an application of this second method. The values secured represent, at all temperatures, the duration of the absolute refractory period in the fibres which recover most quickly. The weight of recent evidence appears to indicate that all medullated fibres have the same absolute refractory period (4,5); if this is true the values here given must hold for all such fibres.

Although many determinations of the duration of the absolute and relative refractory period in nerve have been made either by the muscular summation method (6,7,8,9,10,11,12,13,14,15), or by the method of second electrical response (11,16,17,18,19,20,21,4,5), there have been few systematic studies of the effect of temperature upon duration. Using the muscular summation method, Bazett (7) applied the stimuli directly to the muscle, but believed that, in non-curarized preparations, the refractory period measured was that of the intramuscular nerves; he secured readings at temperatures ranging from 1° to 15° C. Adrian (9,11), by the same method, but stimulating the nerve directly, concerned himself mainly with the effect of temperature upon the form of the whole recovery curve and in most cases did not directly determine the duration of the absolute refractory period. By the method of second electrical response no systematic study of this relationship has been made since the work of Gotch (18) who reported values between 3° and 12° C. only. Other investigators have worked at higher temperatures but have not been directly interested in the effect of temperature itself, and have often failed to specify at what temperature their work was done.

Working at low temperatures Lucas (18) reported that even in fresh preparations, the least interval between stimuli which just gives a summated muscular contraction is definitely longer (20–25 p.c.) than the least interval required for the appearance of a second electrical response in nerve, indicating that a second action potential wave, subnormal in size, may be present in the nerve before it is able to affect the muscle. Adrian (11) finds that this difference is not always present. Kato, Hayashi and Takeuchi (15) submit evidence that this disparity in time does not exist above 15° C., but their argument is somewhat indirect. On the whole it appears that direct measurements of the electric response in the nerve must give the more trustworthy values for refractory period.

It must, however, be recognized that even this method fails to give absolute values for several reasons. Forbes, Ray and Griffith (19) have pointed out that, if the second stimulus be made sufficiently strong, the absolute refractory period, in the classical sense, may be reduced to zero. Such stimuli, while entering the nerve in the period which Lucas (22) once

proposed to designate as "irresponsive" may endure for such a time, or may produce local effects of such a magnitude that the nerve is stimulated after an interval. In such a case the stimulus interval is not a true measure of the "irresponsive" period, or response interval, which they suggest as a new definition of absolute refractory phase. Even this definition must be qualified, however, when the response interval is determined from string galvanometer or oscillograph records, and measured at some distance from the site of stimulation, since it is known that the second action potential wave suffers a retardation in speed of conduction when travelling in the relative refractory phase of the first (16, 19, 23). The response interval therefore appears to be longer than when measured directly under the stimulating electrodes. Measurements of the latter sort have so far been made only through the use of the cathode ray oscillograph (21); it would appear that great accuracy in such readings is not possible (4).

The values here reported approximate the absolute refractory period, in the classical sense, but must depart from the true value of the "irresponsive" period, as discussed above, by an error determined by the strength and duration of the second stimulus, and by these alone. The retardation in conduction time between the stimulating and lead-off electrodes does not introduce any error in the method here used, since, for the short intervals involved, the galvanometer deflection approximates to a ballistic summation of both action potential waves. The response interval is not, and cannot be, measured.

Concerning the magnitude of the error introduced by the second stimulus it is possible to make an approximate estimate. Through the courtesy of Mr B. H. C. Mathews I have obtained oscillograph records of the form of the break shocks delivered by the coreless induction coils used in the present study. He obtained these records under conditions approximately similar to those of these experiments. The two coils give similar results. For the second stimulus the time to maximum is about $0{\cdot}12\sigma$, with a total duration of about $0{\cdot}28\sigma$. It is not possible to know at what time, within this period, stimulation occurs, but the difference between the true value of the response interval and the known stimulus interval can hardly be more than the total duration of the second stimulus, and is probably considerably less. Since the magnitude of the error within these limits cannot be determined, and since the error in the determination of the stimulus interval itself may be as large as $0{\cdot}1\sigma$, I have made no attempt to apply a correction factor to the data. At least it can be stated that the values were secured by a uniform procedure at

all temperatures, and the presence of a systematic error should operate to shift the whole curve slightly along the time axis, without materially altering its form.

Throughout these determinations the first stimulus was set to be somewhat supermaximal; the second stimulus was then made ten times as strong. Under these conditions a least interval is obtained which is not further shortened by a considerable increase in the magnitude of the second stimulus (15); this constant value may be considered as the closest approximation to the true value of the absolute refractory period which can be obtained by this method. The reduction of that period to zero, as described by Forbes, Ray and Griffith (19), is observed only when the second stimulus is made very much stronger, and local effects become very highly developed.

In Fig. 1 are given the results of 34 determinations of the least interval,

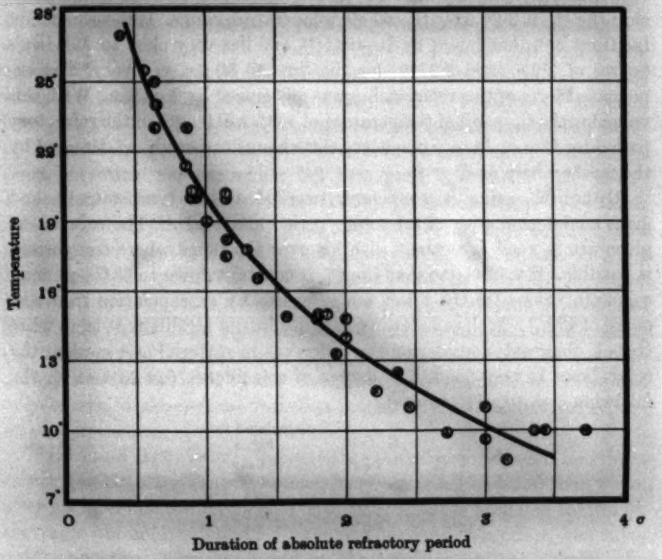

Fig. 1. Influence of temperature upon the duration of the absolute refractory period in the sciatic nerve of the frog. 34 determinations made on 13 nerves from eight animals. The values secured from each nerve are numbered, in the order in which the experiments were made.

carried out on 13 nerves from eight animals (large *R. esculenta*). The values secured from each of the 13 nerves are indicated by the numerals enclosed within each circle; the nerves are numbered in the order in which the experiments were made. Of the eight animals six (nerves 1–10) had previously been kept in the ice-box for several months, at a temperature slightly above 0° C.; the other two (nerves 11–13) had previously been kept in an open tank on the roof, at summer temperatures. Since all of the values group together along one curve it appears that this difference in previous history has no effect upon the value of the absolute refractory period.

Although the values are quite consistent there is sufficient variation to make any mathematical formulation of the results open to some question. As a first approximation a simple exponential curve seems to give a reasonably good fit. The curve drawn through the points in Fig. 1 is of this type[1]. If we accept it as giving a correct picture of the relationship the Q_{10} is 3·06 over the whole temperature range. This value is not far from 3·6, that found by Bazett(7), and lies very close to Adrian's figures of 2·9(9), and 3·27(11) for the time to 50 p.c. recovery. The exponential form of this relationship was recognized by Adrian. With this value for the Q_{10} a fall of temperature of 1° C. will lengthen the refractory period by 12 p.c. In any one nerve this change can easily be detected by the method here used.

Gotch(16), using *R. temporaria*, worked at low temperatures, and gives no determination of refractory period above 12° C. The values here given are in good agreement with his over the range where comparison is possible. If we assume that the Q_{10} is constant down to 3° C., we may calculate values for the lower temperatures by extrapolation from the curve of Fig. 1. Such calculations give results not far different from what Gotch observed; considering the difference in material and method the coincidence is very good. The degree of coincidence can be seen in the following comparative values:

	Gotch	Calculated from Fig. 1
3° C.	7·5σ	6·8σ
4°	7·0	6·0
5°	6·5	5·4
8°	3·5	3·8
12°	2·0	2·4

[1] The equation for this curve is:

$$x = 9{\cdot}4\,(1{\cdot}118)^{-y},$$

where x = refractory period in σ,
y = temperature in degrees Centigrade.

By the method of second electrical response Adrian(11) located the value between 2 and 2·5σ at 13° C. Fig. 1 gives 2·2 for this temperature.

With the method used in the present work it is difficult to secure satisfactory values above 25° C. and below 10° C. As the temperature rises the maximal deflection of the galvanometer is progressively reduced, until the error in reading renders accurate determinations impossible. On the other hand, the increase in maximal deflection which is taken to indicate the presence of a second action potential wave becomes less and less abrupt as the temperature falls, until finally the least interval cannot be located with any certainty.

Field and Brücke(20) have recently reported that between 15° and 19° C. the absolute refractory period for the same material (sciatic nerve of *R. esculenta*), measured by another method, varies from 0·8 to 2·25σ. Erlanger, Gasser and Bishop(4) have published values for *R. catesbiana* which vary from 0·73 to 1·77σ. The temperatures are not given. In both cases the impression given by the values is one of considerable variability in the material. I am inclined to believe that this is not correct. In my experience the behaviour of the nerves is quite uniform, when the temperature is carefully controlled. In addition to the data here given, in which a special attempt was made to determine the value of the least interval accurately, I have secured many other determinations, on other nerves, which fall into good agreement with these. I therefore incline to the opinion that the variation observed by these authors probably arose mainly from temperature differences rather than from variations in the tissues. It might be stated, from an inspection of Fig. 1, that between 15° and 19° C. the value of the least interval varies between 1 and 2σ. Such a description, while strictly correct, gives a false impression of the data, since the experimental points fall closely along the mean curve over the whole range. Of the total variation approximately two-thirds is due to the changes in temperature themselves, and only one-third arises from variation in the material, and other experimental errors.

Field and Brücke also indicate that the refractory period lengthens rather quickly after the preparation has been made and find that when, after an interval, the second nerve of the animal is prepared for study, the value obtained is longer. I have not observed this effect, but have, on the contrary, been impressed by the constancy of the value of the least interval over a considerable time, when temperature remains constant and the stimulation is relatively infrequent, and by single and double shocks only, as in all of the determinations here reported. I have

also found it possible to carry out a series of determinations at various temperatures, and then secure (as did Adrian(9) with the muscular summation method) a final reading at the initial temperature which is almost identical with that previously obtained.

Summary.

The influence of temperature upon the absolute refractory period of the sciatic nerve of *R. esculenta* has been studied. Over the temperature range examined (9°–27° C.) the experimental values outline a curve which is approximately exponential in form. The Q_{10} is 3·06.

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A CRITICISM OF THE HILL-HARTREE METHOD OF CURVE ANALYSIS.

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In the course of an attempt to adapt the method of curve analysis devised by Hill and Hartree(1) for their myothermic studies to the solution of curves of galvanometer deflection given by single action potential waves in nerve (see Amberson and Downing(2)), I have found it necessary to make a critical examination of the validity of this method. This study has led to the detection of certain errors in the procedure previously used in such analysis, and, finally, to the discovery that determinate solutions have not been secured in the older studies. Much of this criticism has taken form as the result of a correspondence between Prof. Hill, Mr Hartree, and myself, in which the various points at issue have been discussed at some length.

Indeed it is Mr Hartree, rather than myself, who is responsible for the discovery of the indeterminate character of the older solutions. In the course of our correspondence he was able to show that a certain analysis which I had submitted was not the only possible solution, but that another and radically different solution could be secured. I therefore was led to re-examine some of the older data, and have found that other solutions can be secured in every case which I have studied. It would now appear that the time relations of heat production in muscle, during and after stimulation, have not been, and presumably cannot be, accurately determined by this method, at least with such galvanometer deflections as have previously been secured. This discussion of the difficulties inherent in this method of curve analysis is published in the hope that the experimental arrangements necessary for the securing of really accurate information may be more clearly seen by those who are interested in such studies.

The basic difficulty in the analysis of curves of galvanometer deflection given by heat produced in nerve or muscle lies in the fact that such

curves rise too slowly toward their maximum. This characteristic is not due to the galvanometer itself, but arises from the fact that there is a considerable delay in the appearance, at the thermocouples, of the heat produced in the tissue. This heat must pass through the tissue itself, and through a sheet of insulation over the "hot" junctions. As a result the curves leave the base line so slowly that the first few ordinates used in the analysis are sufficiently small to permit quite a latitude in the choice of values to be assigned to each block of the analysis. The situation is, therefore, very different from the problem of tide and sound wave analysis into several constituent sine curves, since in such records the curves depart sharply from the physical zero.

It would appear that, in the previous analyses, it has been the practice to assign all of the heat which can possibly be allotted to each block in succession. At least I early adopted this procedure in my own work. This method apparently gives consistent results, and leads to values which certainly constitute a possible solution. For months I proceeded to analyse in this manner, securing solutions which seemed to me to be perfectly determinate, and remaining quite unaware of the wide latitude of choice possible in the assignment of values. Only more recently, and as a result of the above-mentioned discussions, has the whole range of possibilities in analysis become clear.

The wide range of possible solutions can best be made clear by the consideration of an actual example. For this purpose we may examine the problem by which Hill and Hartree originally sought to explain the nature of their method. The data are taken from pp. 102–104 of the paper cited above. Starting with the control curve reproduced in Table I of the present paper they proceeded to calculate the form of galvanometer deflection which would be given by the same heat, taken as unity, when distributed in the manner shown in Fig. 1 (their Fig. 8). The calculated deflection for such a distribution is shown in the series of values for the "observed" deflection given in the second row of Table I. Having thus synthesized a curve they then proceeded to analyse it by the subtraction, in succession, of the same series of ordinates which they had used in the construction of the curve. (See p. 104 of their paper for details.) This analysis must, of course, represent a possible solution of the "observed" curve. It remained to be seen whether it represented the only possible solution. An examination of this example was made, and it was found that, within the limits of error allowable in such work, quite a different solution could be obtained. In Table I the details of an alternative solution of this curve are given. No remainder greater than ± 2 is

TABLE I. Alternative solution of example given by Hill and Hartree.

Time (sec.)	0	0·2	0·4	0·6	0·8	1·0	1·2	1·4	1·6	1·8	2·0	2·2	2·4	2·6	2·8	3·0
Control (C)	0	1	6	29	73	131	191	254	310	358	399	432	455	473	486	494
Observed	0	0	2	13	35	67	104	149	200	251	301	348	386	418	445	465
At 0, 0·4 C.	0	0	2	12	29	52	76	102	124	143	160	173	182	189	194	198
		0	0	1	6	15	28	47	76	108	141	175	204	229	251	267
At 0·2, 0·125 C.		0	0	1	4	9	16	24	32	39	45	50	54	57	59	61
			0	0	2	6	12	23	44	69	96	125	150	172	192	206
At 0·4, 0·125 C.			0	0	1	4	9	16	24	32	39	45	50	54	57	59
				0	1	2	3	7	20	37	57	80	100	118	135	147
At 0·6, 0·085 C.				0	0	1	2	6	11	16	22	26	30	34	37	39
					+1	1	1	1	9	21	35	54	70	84	98	108
At 0·8, 0·085 C.					0	0	1	2	6	11	16	22	26	30	34	37
						+1	0	−1	3	10	19	32	44	54	64	71
At 1·0, 0·085 C.						0	0	1	2	6	11	16	22	26	30	34
							0	−2	1	4	8	16	22	28	34	37
At 1·2, 0·11 C.							0	0	1	3	8	14	21	28	34	39
								−2	0	+1	0	+2	+1	0	0	−2

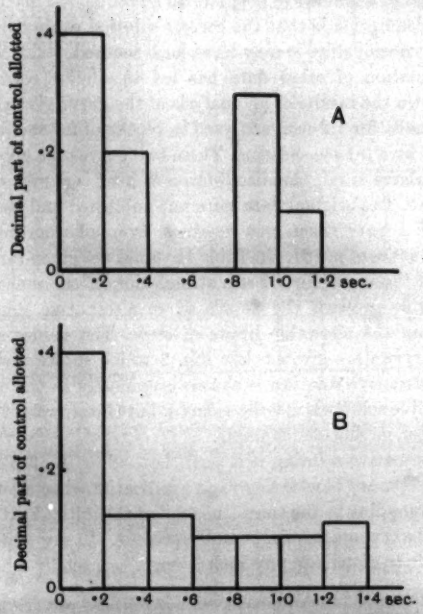

Fig. 1. *A*. Reproduction of example given by Hill and Hartree (this Journ. 54. p. 102. 1920).
B. Alternative solution of example. Details of the analysis are given in Table I.

found in any column[1]. Such remainders are quite within the experimental error of reading the ordinates, and are not significant. While at first glance this solution might be considered less plausible than that given by Hill and Hartree, since they have no remainders whatsoever, this apparent perfection arises merely from the fact that they were taking the curve to pieces in exactly the same way in which they built it up. The alternative solution here presented shows remainders of the size usually found in such analyses; when within the limits specified such remainders do not constitute an objection to the solution.

Other alternative solutions of this same "observed" curve can be obtained. Indeed the number of different answers which can be found appears to depend only upon the ingenuity and patience of the calculator, and, it might be added, upon his possession of sufficient scepticism to distrust any of the solutions as giving *the* proper answer to the problem. Since a number of answers are possible, all equally good mathematically, the fairest presumption is that the correct solution cannot be identified, even though, conceivably, it may have been secured.

An examination of other data has led to similar conclusions. In Fig. 2 are shown the results of an analysis of the curve given by a stimulation of a muscle for 1·2 sec., analysed in blocks of 0·2 sec. by a control curve secured by a 0·1 sec.-heating. These two curves are given on p. 101 of the paper above cited; the distribution of heat is shown on p. 110 of the same paper. The original data were not published and have not been available, but I have taken new readings from photographic enlargements of the curves of p. 101. In Table II values are given for the control curve (*C*) and the curve for 1·2 sec. stimulation of the muscle (*A*). The rest of the table presents the details of an alternative solution which proceeds within the allowable limits of error. The values assigned by Hill and Hartree are given below Fig. 2 which shows their graphical form. The alternative solution is shown graphically in the same figure. Other alternative solutions may be secured, but the argument is probably sufficiently clear in this one example.

In this alternative solution it is seen that, while the heat appears to occur in two separate blocks there is no indication that a sudden burst of heat, corresponding to the third "phase" of the initial heat production of the Hill-Hartree analysis, is really present. In my experience it is always possible to distribute any such "burst," especially when it occurs

[1] In these calculations I have invariably called any decimal part greater than 0·5 as unity, and have discarded all decimal parts less than 0·5. Thus 101·6 becomes 102 and 29·2 becomes 29.

in the later blocks of the analysis, so that the heat falls more uniformly over a number of blocks. The third "phase" of the initial heat production is, therefore, not certainly present, and may be entirely a myth. All analyses show heat production during relaxation, but this heat cannot certainly be distinguished as a separate and distinct phase, sharply localized in time.

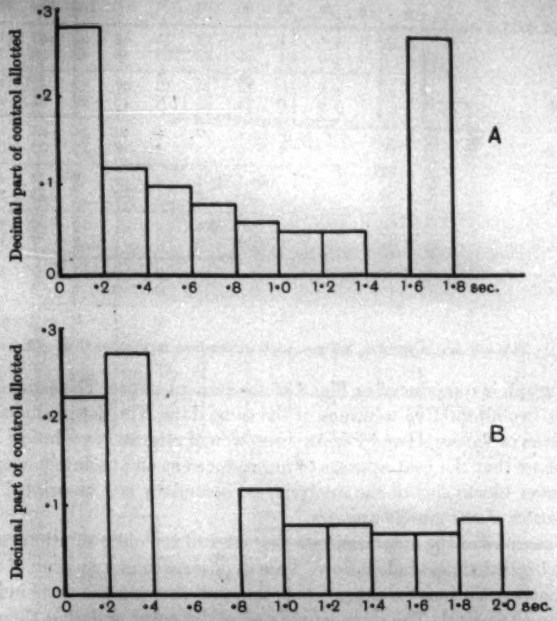

Fig. 2. *A*. Results of analysis given by Hill and Hartree (this Journ. 54. p. 110. 1920).
B. Alternative solution. Details of the analysis are given in Table II.
Assignments of values by Hill and Hartree were read as follows:

Sec.	0	0·2	0·4	0·6	0·8	1·0	1·2	1·4	1·6
	0·28	0·12	0·10	0·08	0·06	0·05	0·05	0	0·27

One more example may be given of the manner in which alternative solutions may be secured. I have examined the data published by Hartree(3) in a paper primarily concerned with setting forth the technique of analysis. In his Fig. 1, on p. 268, he has represented graphically the results secured in an analysis whose details are presented on p. 267.

TABLE II. Alternative solution of Curve *A* of Fig. 7 (Hill and Hartree, this Journ. 54. p. 101. 1920).

Time (sec.)	0	0·2	0·4	0·6	0·8	1·0	1·2	1·4	1·6	1·8	2·0	2·2	2·4	2·6	2·8	3·0
Control (*C*)	0	4	14	44	103	190	290	396	500	603	700	779	838	888	927	956
Observed	0	1	4	14	35	70	118	176	240	309	385	462	540	617	690	757
At 0, 0·225 C.	0	1	3	10	23	43	65	89	113	136	157	175	189	200	209	215
		0	1	4	12	27	53	87	127	173	228	287	351	417	481	542
At 0·2, 0·275 C. (At 0·4 & 0·6, 0 C.)		0	1	4	12	28	52	80	109	137	166	192	214	230	244	255
			0	0	0	−1	1	7	18	36	62	95	137	187	237	287
At 0·8, 0·12 C.					0	0	1	5	12	23	35	48	60	72	84	93
						−1	0	2	6	13	27	47	77	115	153	194
At 1·0, 0·08 C.						0	0	1	4	8	15	23	32	40	48	56
							0	1	2	5	12	24	45	75	105	138
At 1·2, 0·08 C.							0	0	1	4	8	15	23	32	40	48
								+1	1	1	4	9	22	43	65	90
At 1·4, 0·07 C.								0	0	1	3	7	13	20	28	35
									+1	0	1	2	9	23	37	55
At 1·6, 0·07 C.									0	0	1	3	7	13	20	28
										0	0	−1	2	10	17	27
At 1·8, 0·085 C.										0	0	1	4	9	16	25
											0	−2	−2	+1	+1	+2

This solution is valid to 3·8 sec., with remainders no greater than ±2.

This graph is reproduced in Fig. 3 of the present paper. The same figure shows two alternative solutions of the same data. The detailed analyses are given in Tables III and IV. An inspection of alternative solution No. 1 will show that the heat appears to be produced at an absolutely constant rate over blocks 2–8 of the analysis; no secondary rise associated with relaxation of the muscle appears.

Examples of the indeterminate character of the older solutions might be multiplied almost indefinitely. I am in possession of a number of other alternative solutions for other sets of data, all showing the same indeterminate character[1]. The same experimental difficulty underlies them all;

[1] These include alternative solutions of the data of Hartree (Journ. Physiol. 61. p. 255. 1926). One alternative solution of the data given on p. 260 of this paper has the following assignment of values, which may be compared with those chosen by him. Both solutions are equally good mathematically.

Time (sec.)	0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
Hartree's solution	0·12	0·22	0·01	0	0	0·01	0·02	—	0·05	—
Alternative solution	0·16	0·11	0·095	0	0	0	0	0	0·075	0

In both solutions the total heat assigned to the first three blocks of the analysis is nearly the same, but it would appear that it is not possible accurately to know from such records what the distribution of heat is, within this 0·3 second period.

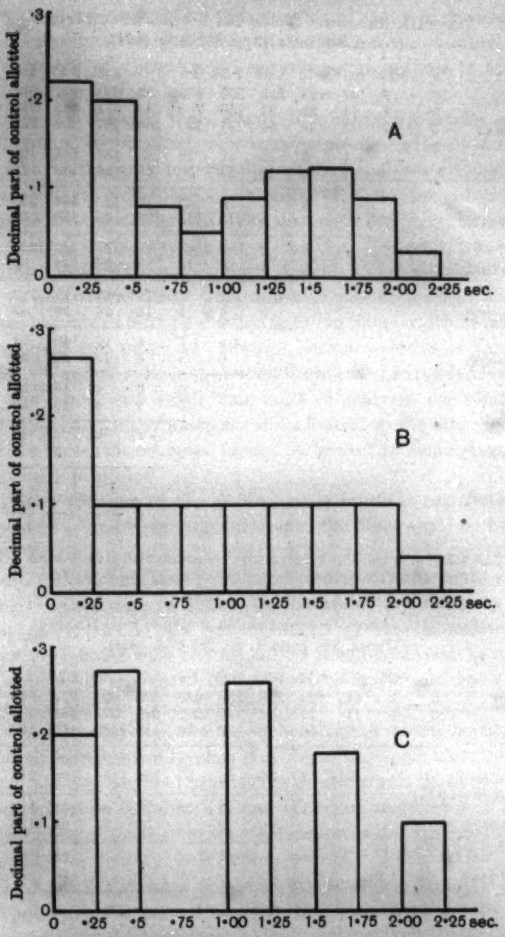

Fig. 3. *A*. Results of analysis given by Hartree (Journ. Sci. Insts. 1. p. 268. 1924).
B. Alternative solution No. 1. Details of the analysis are given in Table III.
C. Alternative solution No. 2. Details of the analysis are given in Table IV.

TABLE III. Alternative solution No. 1 of data from Hartree (Journ. Sci. Insts. 1. pp. 267–268. 1924).

Time (sec.)	0	0·25	0·5	0·75	1·00	1·25	1·5	1·75	2·00	2·25	2·5	2·75	3·0
Control (*C*)	0	7	44	128	238	360	482	595	694	777	844	896	936
Observed	0	1	11	39	83	140	208	285	371	463	555	644	725
At 0, 0·269 C.	0	2	12	34	64	97	130	160	187	209	227	241	252
		−1	−1	5	19	43	78	125	184	254	328	403	473
At 0·25, 0·10 C.		0	1	4	13	24	36	48	59	69	78	84	90
			−2	1	6	19	42	77	125	185	250	319	383
At 0·5, 0·10 C.			0	1	4	13	24	36	48	59	69	78	84
				0	2	6	18	41	77	126	181	241	299
At 0·75, 0·10 C.				0	1	4	13	24	36	48	59	69	78
					+1	2	5	17	41	78	122	172	221
At 1·00, 0·10 C.					0	1	4	13	24	36	48	59	69
						+1	1	4	17	42	74	113	152
At 1·25, 0·10 C.						0	1	4	13	24	36	48	59
							0	0	4	18	38	65	93
At 1·5, 0·10 C.							0	1	4	13	24	36	48
								−1	0	5	14	29	45
At 1·75, 0·10 C.								0	1	4	13	24	36
									−1	1	1	5	9
At 2·00, 0·04 C.									0	0	2	5	10
										+1	−1	0	−1

This solution is valid to 4·0 sec., with remainders no greater than ±1.

TABLE IV. Alternative solution No. 2 of data from Hartree (Journ. Sci. Insts. 1. pp. 267–268. 1924).

Time (sec.)	0	0·25	0·5	0·75	1·00	1·25	1·5	1·75	2·00	2·25	2·5	2·75	3·0
Control (*C*)	0	7	44	128	238	360	482	595	694	777	844	986	936
Observed	0	1	11	39	83	140	208	285	371	463	555	644	725
At 0, 0·2 C.	0	1	9	26	48	72	96	119	139	155	169	179	187
		0	2	13	35	68	112	166	232	308	386	465	538
At 0·25, 0·274 C.		0	2	12	35	65	99	132	163	190	213	231	246
(At 0·5 & 0·75, 0 C.)													
			0	+1	0	3	13	34	69	118	173	234	292
At 1·00, 0·26 C.					0	2	12	33	62	94	125	155	180
(At 1·25, 0 C.)													
						+1	+1	1	7	24	48	79	112
At 1·5, 0·18 C.							0	1	8	23	43	65	87
(At 1·75, 0 C.)													
								0	−1	1	5	14	25
At 2·0, 0·10 C.									0	1	4	13	24
										0	+1	+1	+1

This solution is valid to 4·0 sec., with remainders no greater than ±1.

the curves rise so slowly that a wide latitude of choice in the assignment of values is possible. Determinate solutions will only be obtained through the development of a more rapid recording system, able to follow the thermal events with less delay.

It might be thought that, although solutions in blocks of 0·2 or 0·25 sec. cannot be obtained, it would be possible to analyse in larger blocks. I have attempted to carry through such analyses without success. It is possible to get good solutions, in blocks of 0·5 or 1 sec., of such simple problems as that given by Hartree on pp. 271–272 of the paper just cited, if analysis is made by the use of "built-up" controls, whose nature is described below. It is not similarly possible to get solutions of muscle and nerve heat distribution. This difficulty probably arises from rapid changes in the rate of heat production in living tissue which so complicate the form of the galvanometer deflection that no solution in large blocks is possible. We seem to be caught upon the horns of an insoluble dilemma. On the one hand, with small time units of analysis, the solutions are indeterminate, and many answers can be found; on the other hand, with longer time units, the analysis cannot be pushed to completion, and no full solution is possible.

My initial criticism of the method arose from the conviction that a systematic error had been introduced into the older analyses by the use of control heatings enduring for less than the time unit of analysis. Since this point will become more and more important the more rapid the recording it seems wise to discuss the nature of this difficulty. Of the older analyses it may be said, not only that they were indeterminate, but that they were made with control curves which could not have given a correct solution in any case. In the previous examples I have used the same data which Hill and Hartree have used, including the control curves, which were always secured by heating the muscle for periods less than the time unit of analysis. With "built-up" controls, now to be described, another series of alternative solutions can be obtained. Thus the observed curve of Tables III and IV, when analysed with a "built-up" control, may, in one solution, be interpreted in terms of three blocks of heat only, as follows: 0–0·25 sec., 0·404 C.; 0·75–1·00 sec., 0·28 C.; 1·5–1·75 sec., 0·32 C. Other solutions are possible. From the point of view of securing the correct answer, therefore, such controls are no improvement over the older type, so long as the galvanometer deflections rise slowly. Under certain circumstances, however, where the galvanometer movement is rapid enough to follow relatively slow changes in heat production, and where analysis may proceed in time blocks so long that

the initial ordinates are large, and permit only one answer, these "built-up" controls, or their experimental equivalent, are absolutely necessary for securing even an approximately correct solution, and will become increasingly important with further improvements in technique.

I was led to suspect the existence of such an error by the observation that, with a control heating enduring for 0·05 sec. only, Hartree was not able, in the example given on pp. 271–272, to allot anything like the correct value of heat to the first blocks of his analysis, and was left with a remnant of heat after the conclusion of the known periods of heating. I therefore constructed a new "built-up" control curve by combining five curves as given by the 0·05 sec. heating, spaced at intervals of 0·05 sec. For this calculation it was necessary to interpolate values to 0·05 sec. intervals. By dividing all the ordinates thus obtained by five I was able to secure a new control curve, representing the form of galvanometer deflection which would be given by the same control heat when distributed over 0·25 sec., *i.e.* a period identical with the time unit of analysis. This new "built-up" control appeared to give a much better analysis, with the possibility of assigning a more nearly correct amount of heat to the first blocks of each of the two known periods of heating, and the elimination of the small heat remainders after each period. I have since found that, by juggling the figures, it is possible to get a somewhat different answer, but this problem appears to give a more nearly determinate solution than is usual when analysis proceeds in small time blocks, probably because the heat production is absolutely uniform in each of the two periods.

Concerning the validity of my original criticism there still exists some difference of opinion among us. However, in recent work, Prof. Hill has secured results which he considers satisfactory by a method identical with that adopted by me. The nature of the error introduced into the analysis by the old procedure may be made clear by a simple example. For this purpose we may examine the differences in the results obtained by the use of a control curve in which the heating endured for the same period as the time unit of analysis, as compared with those given by a control in which the heating endured for only half as long. Hill(4) has just published a study of the heat-production in crustacean nerve, using control heatings enduring for the same period as the analysis block. In this work the first ordinate used in the analysis is so large that only one solution is possible, and the objections above discussed do not apply. On p. 166, Fig. 5, Hill gives the form of galvanometer deflection produced by a control heating enduring for 0·1 min. (Curve *b*). The analysis of

Curve *a* by this control is shown in the same figure; the details of the analysis are given on p. 168. From the curves given it is possible to make an approximate estimate of the form of deflection which would be given by the same amount of control heat if it were generated in 0·05 min. instead of 0·1 min. An analysis of the first part of the observed curve by such a calculated control is given in Table V. It may be compared

TABLE V. Solution of data from Hill (Proc. Roy. Soc. B, 105. p. 168. 1929) by "instantaneous" control.

Time (min.)	0	0·1	0·2	0·3	0·4	0·5
Control (*C*)	0	1066	1100	861	645	490
Observed	0	404	694	782	862	928
At 0, 0·379 C.	0	404	417	326	244	186
		0	277	456	618	742
At 0·1, 0·26 C.		0	277	286	224	168
			0	170	394	574
At 0·2, 0·159 C.			0	170	175	137
				0	219	437
At 0·3, 0·205 C.				0	219	226
					0	211
At 0·4, 0·198 C.					0	211
						0

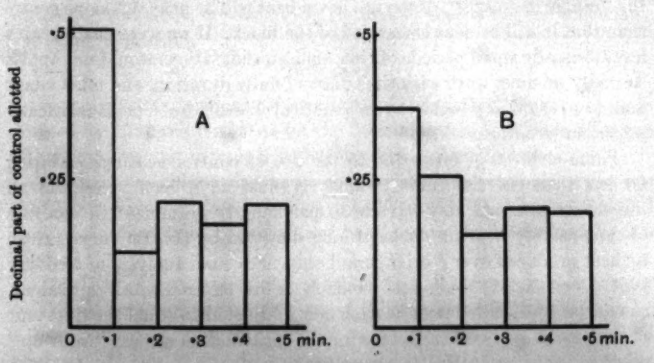

Fig. 4. *A*. First portion of analysis given by Hill (Proc. Roy. Soc. B, 105. p. 168. 1929). *B*. Solution of the same observed curve by the use of a calculated control curve which would be given if the control heat had endured for 0·05 min. only, *i.e.* one-half of the time unit of analysis. Details of the analysis are given in Table V. The distortion of the analysis produced by the use of such an "instantaneous" control is well shown. With a really instantaneous control heating the error would be even greater.

with the results given by a 0·1 min. control curve by inspection of Fig. 4.

The older procedure appears to have arisen from the belief that the shorter the control heating the closer would be the approach to the theoretically correct method, which would be completely attained only through the use of an absolutely instantaneous control heating. It is now seen that this belief was not correct. If, for practical reasons, it becomes necessary to use blocks of finite duration in the analysis, it is equally necessary to prolong the control heating so that it endures for the same period as the time unit of analysis. If this is not done the control curve rises more rapidly than it should. As a result less heat is allocated to the first block of the analysis than is correct, and more heat remains to be disposed of in later blocks. The resulting distortion of the curve is well shown in Fig. 4.

The validity of the new type of control may be established in a number of other ways which I shall not attempt further to detail. It will probably be sufficiently obvious that, in the absence of any information concerning the distribution of the unknown heat within each block of the analysis, it is best to use a control heating whose "centre of gravity" coincides with the centre of each analysis block. It is certainly more probable that the "centre of gravity" of the unknown heat will lie near this same centre than that it will be near either end of the block. If we were dealing with infinitesimally small periods of time such considerations would not apply. Actually we must work with time units of finite duration, and must adapt ourselves, so far as possible, to the practical exigencies of a mathematically imperfect situation.

Some element of error due to the use of control heatings enduring for less than the time unit of analysis must have been present in all the older work, and may have been particularly prominent in analyses of the recovery heat in blocks of long duration by control curves given by heat produced over a brief period only. For such analyses of recovery heat a calculated "built-up" control, or its experimental equivalent, should be used, the control heating being adjusted to equal the time unit of analysis in every case. Details for the construction of such "built-up" controls are given in Hill's recent paper.

In this connection it may be pointed out that the analysis of recovery heat in 5-sec. blocks using control curves given by heat produced in a very much briefer period, after the method used by Hartree and Hill(5) in a later study, may be entirely responsible for the apparent rise of the recovery heat to a maximum after 30–40 sec. They had "expected the

chemical processes associated with oxidative recovery to start at a high level, and to decline continually to zero." An analysis of this data with a "built-up" control of the proper length may show that their expectation was not unwarranted.

Some appreciation of the difficulties which I have here described must have been in the mind of Gerard(6) when he developed his "method by difference" for the analysis of nerve heat records. Although adopted for quite other reasons, this method appears to go very far toward meeting the objections which I have here outlined against the "instantaneous" controls, since he analysed out the major part of the heat produced during stimulation by a control curve produced by a heating equal in duration to the stimulation time. Even here, however, some difficulties remain. I am convinced that, even with such modifications in procedure as he has introduced, or I have suggested, and with records in which only one answer is possible, the results of any analysis must be accepted only with great caution.

It must be emphasized, in conclusion, that the difficulties here described do not at all affect the values for total heat, either for the initial or for recovery heat, or any inferences or calculations which have been made from them. All solutions give the same values for total heat, whose determination depends upon the area of the curves, for which only one value is possible.

Summary.

A critical examination of the validity of the method of curve analysis devised by Hill and Hartree for the determination of the time relations of heat production in their myothermic studies has shown:

(1) That the older solutions do not give the only possible answer, but that alternative solutions can always be obtained. The time relations of heat production have, therefore, not been accurately determined by this method.

(2) That, where determinate solutions can be secured, it is important to use, as a control curve, the form of galvanometer deflection given by a control heating enduring for the same period as the time unit of analysis. If this is not done a systematic error is introduced into the analysis.

Values for total heat, both initial and recovery, are not affected by this criticism.

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1. Hill and Hartree. This Journ. 54. p. 84. 1920.
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3. Hartree. Journ. Sci. Insts. 1. p. 265. 1924.
4. Hill. Proc. Roy. Soc. B, 105. p. 153. 1929.
5. Hartree and Hill. This Journ. 56. p. 367. 1922.
6. Gerard. This Journ. 62. p. 349. 1927.

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THE ANALYSIS OF GALVANOMETER CURVES.

By A. V. HILL.

With an Appendix by W. HARTREE.

THE criticism contained in the preceding paper by W. R. Amberson, of the method of curve analysis employed by Hartree and Hill in their work on the heat production of muscles, will be discussed more fully later by W. Hartree, in connection with experimental observations now being undertaken with a much quicker recording system of thermopile and galvanometer than has hitherto been available. Since it may be some time before this investigation is completed, and in order to prevent misunderstanding in the meantime, it is desirable to point out:

(1) That the results of the older analyses, viz. those prior to 1925, which are the only ones, with one exception, criticized by Amberson, were admitted by Hartree(1) in 1925 to be relatively indeterminate, in the following terms: "in view of recent improvements in technique the results then obtained can now be regarded as little better than a first approximation."

(2) That the improvements in technique referred to (*i.e.* in quickness of response of galvanometer and thermopile) made during and since 1925 have rendered the results of the analyses of the heat production of frog's sartorius muscles very far from indeterminate, even when the time-unit of analysis is considerably shorter than that ($\frac{1}{5}$ sec.) adopted in the earlier experiments: this will be shown in detail later by Hartree. The horns of Amberson's "insoluble dilemma" have in fact been dissolved away long since in experimental improvements of the type to which he, somewhat vaguely, refers. Indeed in Hartree's latest experiments (unpublished) the relaxation heat can be seen as an obvious hump on the photographic record (cf. Fig. 2).

(3) That the third phase of the initial heat production (the relaxation heat) is certainly not "entirely a myth": apart from analyses of observations made during and since 1925, which have demonstrated its existence beyond dispute, Hartree's present records exhibit it, *without any analysis*, in Amberson's words, as a "separate and distinct phase,

sharply localized in time." No constant or continuously declining rate of heat production could possibly lead to a second upstroke on the photographic record. We have been perhaps remiss in not giving fuller details of the analyses in our more recent papers, owing to our desire to save space, and expense in printing. This omission is repaired by Hartree in Appendix II, and it will be found that the utmost ingenuity expended even on the 1925 example there given will not conjure away the relaxation heat.

(4) That the suggestion that the use of a short interval of control heating may be "responsible for the apparent rise of the recovery heat to a maximum" is entirely without foundation, as will be shown in detail by Hartree later: the results of the analysis of the recovery heat are quite unaffected by the adoption of the method and of the "new type of control" advocated by Amberson.

(5) That, while the numerical results of the analysis by 0·1 sec., in the case of the tortoise muscle referred to by Amberson in his footnote (p. 72) can admittedly be somewhat varied in detail, the general distribution of the heat into its two phases, contraction and relaxation, will be found to be the same in the two solutions by Hartree and by Amberson respectively.

The reason why the analysis in this case was less sharp than in that of the frog's sartorius given in Appendix II was principally that only one face of the thermopile could be exposed to the biceps cruris of the tortoise. Consequently the rise of temperature of the "hot" junctions took nearly four times as long and the records rose less rapidly.

In all the correspondence between us and him described by Amberson the case referred to was of a curve attaining its maximum in 3 to 4 sec., which was to be analysed in time units of 0·2 (or 0·25) sec. Amberson's contention was that superior results are obtained if the control curve be made by heating for 0·2 (or 0·25) sec. rather than for 0·04 to 0·10 sec., as was customary with us. This contention we did not, and we do not, accept. My personal opinion is that in analysing these older curves the form of the control heating within the 0·2 sec. of the time unit of analysis is a matter of complete indifference. With the newer curves (1925 and since) a shorter time unit (0·1 sec.) is advisable, and has been employed (see (4)), and within this the form of the control heating is again a matter of indifference. With the latest apparatus (now coming into use) a still shorter time unit (0·05 sec.) is advisable during part of the analysis, and again, within this, the form of the control heating is a matter of indifference.

It is true that in recent analyses of the heat production of crustacean nerve(2) the period of control heating, and of analysis, were both made the same as that of stimulation. The arrangement, however, is really nothing but a simple modification of the method of Gerard ((3) p. 354): it avoided the use of more than one control curve. The essential point about it is *not* that the time unit of analysis is equal to that of the control heating, but that *from the numbers representing the curve to be analysed are first subtracted a similar set of numbers representing a control heating of duration equal to that of stimulation.* This subtraction having once been performed the question of whether the interval of control heating shall or shall not be equal to that of analysis is a matter of indifference, as is shown in Appendix I below. No claim was made that the results obtained by the simple procedure adopted in this special case were in any way superior to those obtained by other methods. Even were they superior, which they are not, in the case of an analysis in 6-sec. time units by a control curve reaching its maximum within 9 sec., it would not follow in the least that the same superiority would exist in the case of an analysis in 0·2-sec. time units by a control reaching its maximum in 3 to 4 sec. The two problems, with such different times, and ratios of times, involved, are essentially different.

SUMMARY.

The criticisms by Amberson of the method of curve analysis adopted by Hartree and Hill, in their work on the heat production of muscle and nerve, are discussed.

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1. Hartree. This Journ. 60. p. 269. 1925.
2. Hill. Proc. Roy. Soc. B, 105. p. 153. 1929.
3. Gerard. This Journ. 62. p. 349. 1927.
4. Hartree. Ibid. 61. p. 255. 1926.

APPENDIX I.

A "control" curve for 1·2 sec. heating of a nerve on a thermopile (see (9)) has the following form:

Time in 1·2 sec.	0	1	2	3	4	5	6	7	8	9	10	11
Deflection in mm.	0	25	66	93	111	121	126	127	124	118	112	106
Time in 1·2 sec.	12	13	14	15	16	17	18	19	20	21	22	23
Deflection in mm.	101	96	91	86	81	76	72	69	65	61	58	54
Time in 1·2 sec.	24	25	26	27	28	29	30	31	32	33	34	35
Deflection in mm.	51	48	45	41	39	36	33	31	28	26	23	21

A "control" curve for 6 sec. heating can readily be constructed from this, as described in (9) p. 169. It has the following form:

Time in seconds	0	6	12	18	24	30	36	42
Deflection in mm.	0	416	607	480	363	272	194	129

It is required to analyse the following curve for 6 sec. stimulation of a crab's nerve.

Time in seconds	0	6	12	18	24	30	36	42
Deflection in mm.	0	40·5	68·0	79·4	87·0	92·6	97·7	102·2

The analysis is carried out in the manner suggested by Gerard ((9) p. 354), viz. "by analysing, instead of the whole curve resulting from, say, a 10-sec. stimulus of the nerve, the comparatively small difference between this and the curve produced by warming with a uniform current for the same time." In the present case for 10 sec. read 6 sec. The following differences are then obtained:

TABLE I.

Time in seconds	0	6	12	18	24	30	36	42
Deflection in mm.	0	40·5	68·0	79·4	87·0	92·6	97·7	102·2
Subtract 40·5/416 of 6 sec. control	0	40·5	59·0	46·7	35·3	26·4	18·9	12·9
Difference to be analysed	0	0	9·0	32·7	51·7	66·2	78·8	89·3

The analysis of the differences in the last line can then be carried out in various ways: the most convenient way (*A*), if no finer details be required, is clearly to use *the same* 6-sec. control curve as was already employed, since *two* control tables have not then to be constructed and used.

TABLE II.

Time in seconds	0	6	12	18	24	30	36	42
Difference to be analysed	0	0	9·0	32·7	51·7	66·2	78·8	89·3
At 6 sec. subtract 9/416 of 6 sec. control, leaving	0	0	0	19·6	41·3	58·3	72·9	85·1
At 12 sec. subtract 19·6/416 of 6 sec. control, leaving	0	0	0	0	12·8	35·7	55·8	72·3
At 18 sec. subtract 12·8/416 of 6 sec. control, leaving	0	0	0	0	0	17·1	38·0	61·1
At 24 sec. subtract 17·1/416 of 6 sec. control, leaving	0	0	0	0	0	0	13·1	41·4
At 30 sec. subtract 13·1/416 of 6 sec. control, leaving	0	0	0	0	0	0	0	22·2
At 36 sec. subtract 22·2/416 of 6 sec. control, leaving	0	0	0	0	0	0	0	0

Clearly Table I and Table II can be compressed into a single Table as was done in (9).

The results are given in *A*, Fig. 1. The broken line is drawn through the results of the analysis to indicate the most probable course of the recovery heat production.

The difference can, however, be analysed in another way, viz. (*B*) by the use of the control curve for 1·2 sec. heating given above.

TABLE III.

Time in seconds	0	6	12	18	24	30	36	42
Difference to be analysed	0	0	9·0	32·7	51·7	66·2	78·8	89·3
At 6 sec. subtract 9/120 of 1·2 sec. control, leaving	0	0	0	24·3	45·2	61·3	75·2	86·8
At 12 sec. subtract 24·3/120 of 1·2 sec. control, leaving	0	0	0	0	22·5	43·9	62·0	77·1
At 18 sec. subtract 22·5/120 of 1·2 sec. control, leaving	0	0	0	0	0	22·9	45·9	64·9
At 24 sec. subtract 22·9/120 of 1·2 sec. control, leaving	0	0	0	0	0	0	24·6	48·5
At 30 sec. subtract 24·6/120 of 1·2 sec. control, leaving	0	0	0	0	0	0	0	25·5
At 36 sec. subtract 25·5/120 of 1·2 sec. control, leaving	0	0	0	0	0	0	0	0

The results are given in *B*, Fig. 1. The broken line, as before, indicates the most probable course of the recovery heat production.

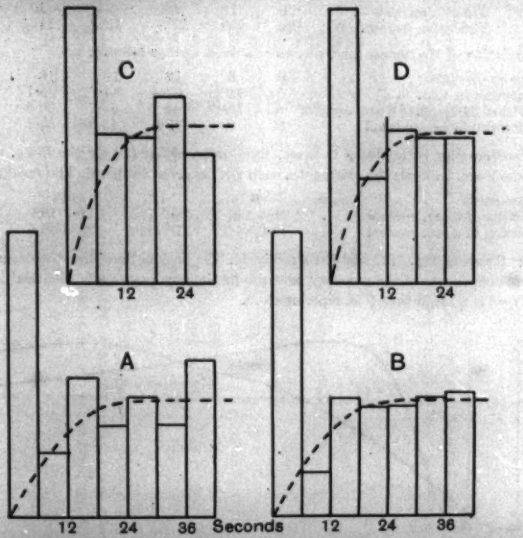

Fig. 1. Analysis of heat production of crab's nerve stimulated for 6 sec.; *A* and *B* alternative analyses of one experiment; *C* and *D* of another. In every case the numbers corresponding to a control curve of the same duration as the stimulus are subtracted from the numbers corresponding to the curve to be analysed (Gerard). The analysis is then conducted in time units of 6 sec. In *A* the control curve used is for 6 sec. heating and in *B* for 1·2 sec. In *C* the control curve is for 6 sec. heating, in *D* for 2 sec. Note that the broken lines, representing the smoothed results of the analysis of the recovery heat, are practically identical in *A* and *B*, and in *C* and *D*, respectively. *A* and *C* are in no way superior to *B* and *D*, as Amberson's paper would suggest.

A comparison of *A* and *B* shows that there is nothing to choose between the smoothed results of the two analyses: the broken lines are the same in both. So far from *A* being the better, it actually shows in this instance rather greater "oscillations" than *B*. Thus the method of analysis adopted in (1) is, in fact, nothing but that of Gerard, with a slight modification for simplicity. The essential point of it is *not* that the interval of analysis is equal to that of the control heating, as advocated by Amberson—this is shown to be of no importance—but that (as Gerard suggested in 1927) a control curve for an interval of heating equal to that of stimulation is subtracted before the rest of the analysis is carried out.

Lest a single example be not regarded as sufficient, a second one has been calculated from the same series of experiments (2). A control curve for 2 sec. heating has the form:

Time in seconds	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Deflection in mm.	0	26	69·5	86·5	90·5	88·5	83	76·5	70	64	58·5	53	48	43	38·5	34

From this a control curve for 6 sec. heating is constructed; it is:

Time in seconds	0	6	12	18	24	30
Deflection in mm.	0	182	262	210½	159½	115½

The stimulation of the nerves for 6 sec. gave a deflection as follows:

Time in seconds	0	6	12	18	24	30
Deflection in mm.	0	34·2	67·7	84·7	95·7	102·8
Subtract 34·2/182 of 6 sec. control	0	34·2	49·1	39·7	30·0	21·6
Difference to be analysed	0	0	18·6	45·0	65·7	81·2

The difference may be analysed in 6-sec. time units, either (*C*) by the 6-sec. control or (*D*) by the 2-sec. control: the arithmetic need not be given in detail. The results are:

Time in seconds	0	6	12	18	24
(*C*) Fraction of 6 sec. control	.	18·6/182	18·2/182	23·2/182	16·0/182
(*D*) Fraction of 2 sec. control	.	18·6/86·5	27·2/86·5	25·8/86·5	25·9/86·5

They are shown in Fig. 1, *C* and *D* respectively. The broken lines drawn to represent the most probable course of the recovery heat production are practically identical in the two cases. There is no sign that *C* is superior to *D*.

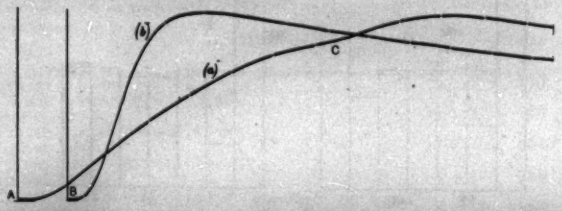

Fig. 2. Experiment of Jan. 1930. (*a*) Record of heat-production of frog's sartorius in response to 0·75 sec. tetanus at 0° C. Stimulus commenced at *A*: time marks every 0·2 sec. (*b*) Control for 0·1 sec. heating, commencing at *B*. Note the "hump" beginning at *C* on curve (*a*), corresponding in time to the onset of relaxation. An analysis of records taken with the same muscle, but with the drum running much faster, gave relaxation heat equal to 33 p.c. of the total initial heat, distributed between 1 and 2 sec. after start of stimulus.

APPENDIX II.

By W. HARTREE

Exp. of March 20, 1925, this being one of the experiments referred to in (1). Frog's sartorii at 0° C. in O_2; 0·1 sec. isometric tetanus; control curve with 0·04 sec. heating. Each record, approximately 100 mm. high, was reduced to a maximum of 1000 and the means taken of three and four, for "stimulus" and for "control" respectively.

Time (sec.)	0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0	1·1	1·2	1·3	1·4	1·5	1·6
Control	0	13	82	201	342	486	614	723	812	877	926	956	978	990	996	998	1000
Stimulus	0	1	16½	55	114	190	275	371	472	571	664	746	817	871	914	945	969
At 0 0·16	.	−1	3½	23	59½	112½	176½	255½	342	430½	516	593	660½	712½	754½	785	809
At 0·1 0·28	.	.	0	0	3½	17	40½	83½	139½	203	270½	334½	393	438½	477½	506	528
At 0·2 0·03	.	.	.	−½	1	11	30½	69	121	181½	246	308	365	410	448	476½	498
At 0·3 0·11	.	.	.	.	0	2	8½	31½	67½	114	167	219	268	308½	343	368½	389
At 0·4 0·09	.	.	.	.	.	1	1	13½	36½	70	112	154	195	229½	260	282½	301
At 0·5 0·15	.	.	.	.	.	.	−½	1½	6½	19	39	61½	87	108	128	143½	157½
At 0·6 0·07	.	.	.	.	.	.	.	½	½	5	15	27½	44	57	71	82½	92½
At 0·7 0·06	.	.	.	.	.	.	.	.	−½	0	3	7	15	20	27½	33½	40
At 0·8 0·03	.	.	.	.	.	.	.	.	.	−½	½	1	5	5½	9	12½	15½
At 0·9 0·02	.	.	.	.	.	.	.	.	.	.	½	−½	1	−1½	−1	½	1

It will be found that the solution given in the left-hand column cannot be altered to any significant extent provided the remainders arising in the analysis be kept as small as possible. It is obvious that there is a large outburst of heat early in contraction, and that about half the total initial heat occurs during relaxation.

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THE TEMPERATURES IN THE TISSUES WHICH ACCOMPANY TEMPERATURE SENSATIONS.

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The rate of penetration of heat and cold into the skin has been considered on theoretical grounds by Goldscheider(7) and by Pütter(11), but no records have been made of temperatures within the tissues coincident with temperature sensations. This paper deals with such measurements.

The results demonstrate that the values previously assumed on theoretical grounds for the rate of thermal conduction are very misleading. A comparison of the latency of sensation with the time taken for heat penetration also allows an approximate calculation of the probable depths of the end-organs concerned. Histological studies attempting to correlate the physiological results with the structures involved are in progress.

When a preliminary communication was made (Bazett and McGlone(1)), it appeared probable that the results might substantiate one of the theories of the mode of stimulation of the end-organs, but the unforeseen complexity of the temperature gradients within the resting skin interferes with a complete interpretation of the data. When the data obtained in the dermis were combined into a composite curve, it became evident that the gradients in this area were much modified by the anatomical arrangement of the blood vessels. These results (many of them obtained by the three of us as control observations before the induction of temperature sensations) have been already published (Bazett and McGlone(2)). The data demonstrated that a reverse temperature gradient can exist normally at depths of about 1 mm. in the skin of the forearm. Consequently no assumption can be made as to the relation of thermal gradients to sensation, nor can the data obtained be utilized to determine the mode of stimulation of the end-organs, until the depth of these end-organs and their relations to blood vessels are first completely established.

Methods employed.

Temperatures were recorded by thermocouples either of the needle type inserted obliquely or of the McGlone wire loop pattern (Bazett and McGlone[2,3]). The electrical connections were as described in these papers. The precautions against parasitic currents described by McGlone and Bazett[9] were taken. The temperature changes were determined by galvanometer deflection from two or three D'Arsonval galvanometers (Leeds and Northrup, type 2500 A) which recorded simultaneously on photographic paper travelling 3 to 5 mm. a second. These galvanometers had undamped periods of 3 to 4 sec. and, when in use, were critically damped. In the most recent experiments simultaneous records have been made from four galvanometers. The additional instrument has been a torsion string galvanometer (Kipp and Zonen, Delft, Holland) of 10 ohm internal resistance, with a period of 0·02 sec.; it is less sensitive and can only be used in connection with thermocouples of low resistance; the use of short thermocouples increases the technical difficulties and heavier wire introduces additional errors. The constant junctions of short thermocouples were placed in a small thermos flask of 25 c.c. capacity maintained at room temperature. The string galvanometer has been employed to check the magnitude of errors from instrumental lag. The error is not great, particularly in estimations of the beginning of the thermal changes, as was demonstrated by simultaneous records of surface temperature changes by both systems and by the data presented in Fig. 1. This error has been neglected.

Cold and warm spots were identified on the extensor aspect of the forearm and thermocouples were introduced obliquely with the thermojunction adjusted beneath a selected spot. In the later experiments McGlone loops were alone used; these consisted of constantan-manganin couples of wire of 60 to 80 μ diameter. Sewing needles (No. 16) about 0·36 mm. diameter, ground to a chisel-shaped point, were passed through the tissue so as to transfix it at the desired depths. The entrance and exit points were usually 5 to 15 mm. distant from the cold or warm spot. Loop couples were then drawn through until the junctions lay beneath the marked spot. An æsthesiometer was brought into light contact with the surface at this spot and the exact position and depth of the couples were determined by X-ray photographs; the positions of the couples were adjusted if necessary and two stereoscopic photographs were taken with the target 1·0 to 1·5 m. distant. Finally a loop thermocouple of the same or heavier wire was adjusted on the surface over the spot to be investi-

gated; the largest size used consisted of constantan and iron wire of approximately 0·2 mm. diameter. The heat capacity of the couples for

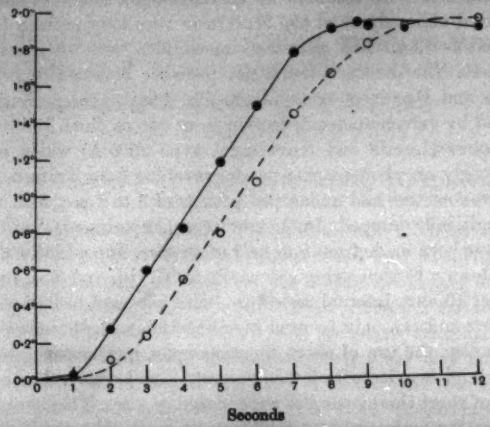

Fig. 1. Ordinates: average change in temperature at 0·9 mm. depth; abscissæ: periods recorded by metronome (0·94 sec.). The solid circles and continuous line represent values (average of 5 stimuli) obtained with the thermocouple connected to a torsion string galvanometer; the open circles and interrupted line, those obtained when connected with a D'Arsonval galvanometer (average of 3 stimuli). The average stimulus for the first series was +7·51° applied for 5·43 sec., for the second +8·06° applied for 5·40 sec. The average latency of the sensation of warmth was 1·17 sec. in the first series and 1·33 sec. in the second. Both warm and cold spots were present.

a rise of 1° C. in 1 cm. of the wire in each direction has been $0{\cdot}9 \times 10^{-4}$ for the smallest, and $6{\cdot}1 \times 10^{-4}$ calories for the largest. The heat conductivity calculated on the basis of 1° C. difference in temperature at a distance of 1 cm. from the junction has varied between $0{\cdot}6 \times 10^{-5}$ and $8{\cdot}1 \times 10^{-5}$ calories per second.

During the period of preparation the bare forearm was exposed to the room conditions for at least 1 hour, and after the introduction of the thermocouples 30 minutes elapsed before recording, in which time any inflammatory reaction was subsiding. Although a "flare" was no longer visible, inflammatory processes along the paths of the needles were probably present.

The æsthesiometer used is of the Thunberg type, and consists of a metal cylinder through which water circulates from thermos-flask

reservoirs; it has interchangeable points. The truncated cones employed had surfaces of 1·5 or 11 mm. diameter. The forearm was fixed on an arm rest, and the æsthesiometer was clamped to a microscope stand, the rack and pinion of which was used in application. With care the point could be applied fairly rapidly with light pressure on to the marked spot, but even with these precautions the pressure and position of the application were not absolutely constant. The time of application was recorded, through the changes in surface temperature, by an electric signal or by a signal operated by air transmission. Its duration was usually about 8 sec. (extreme values 1 to 17 sec.). The subject signalled the duration of sensation (electro-magnet or tambour). Measurement of parallax errors was made from records obtained on stationary paper.

A series of stimulations of the end-organs was made at intervals which were only occasionally less than 5 minutes. During this interval the previous temperature was almost regained; rarely was the return complete. Most of the experiments were made in warm rooms, a few in cold rooms, without any experimental variation of the circulation. The room conditions were carefully measured, and were classified according to the effective temperature (basic scale) of Houghton and his co-workers (for reference, see Bazett and McGlone(2)). A few experiments have been repeated after an inflammatory reaction has been produced in the neighbourhood by scalding or by multiple histamine punctures (Bazett and McGlone(2)).

An abbreviated protocol is given as an example of the general procedure. In this experiment the time from the introduction of the needles to the first stimulation was longer; the intervals between the stimulations were shorter than in any other experiment. It is selected, because, during the long fore-period, inflammatory reactions disappeared.

Exp. 1. The experiment was conducted in a room at 21·0° to 23·9° dry, and 13·1° to 14·9° wet bulb; a dry kata-thermometer gave values of 4·9 to 4·6 and a wet 17·2 to 16·0 millicalories per sq. cm. per sec. as the cooling power. The estimated air movement was 5·4 to 9·6 m. per min. and the effective temperature (basic scale) 16·7° to 18·6°. This environmental temperature was classed as warm. The subject employed was Br.

10·05–10·50. Arm bared, warm spot identified. Around this were four cold spots, the nearest 2·0 mm. distant. Applicator 1·5 mm. diameter.

10·50–15·00. Two loop thermocouples introduced, and X-ray photographs taken. A delay of 2 to 3 hours occurred through instrumental difficulties.

15·00. Records of normal gradients. Surface 31·20°; 0·9 mm. 31·42° and 2·0 mm. 31·56°. Sensitivities of the galvanometers varied between 0·89° and 2·37° per cm. deflection according to the adjustment of external resistance. No hyperæmia noted.

15·07. Stimulation (1) with 29·6° for 8·6 sec. The initial, maximal change and final temperatures were: surface 31·08°, 30·66° and 31·18°; 0·9 mm. 31·32°, 30·96° and 31·32°;

2·0 mm. 31·48°, 31·45° and 31·48°. Final record 38 sec. after end of stimulation. Doubtful sensation of warmth during application and definite warmth on removal.

15·11. Stimulation (2) with 40° for 10·1 sec. The temperatures were: surface 31·27°, >32·5° and 31·48°; 0·9 mm. 31·46°, 32·49° and 31·50°; 2·0 mm. 31·56°, 31·82° and 31·73°. Final record 31 sec. after end of stimulation. Doubtful sensation of warmth.

15·25. Stimulation (3) with 44·5° with more than average pressure. Latency of warm sensation 1·3 sec (see Fig. 2).

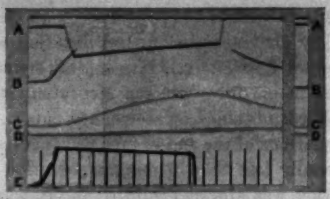

Fig. 2. Exp. 1, stimulation 3 (reduced ⅓). In this and in Figs. 3 and 4 record *A* indicates the duration of sensation (depression of the record); record *E*, the downward movement (upstroke in the record) or upward movement of the applicator (1·5 mm. point). Record *B* was obtained from the surface, and *C* and *D* from 0·9 and 2·0 mm. respectively. To correct for parallax the records *A*, *D* and *E* should be moved in the original record 0·7, 0·3, 0·3 mm. to the right. In this experiment the stimulus was 44·5° applied for 10·1 sec. The initial, maximal and final temperatures were: surface 32·65°, >35·9° and 32·55°; 0·9 mm. 32·7°, 34·45° and 32·7°; 2·0 mm. 32·5°, 32·8° and 32·65°. The sensation was warm. An interval of 28 sec. is omitted. The signal record has been redrawn to economise in space. Time is shown in seconds.

15·32. Stimulation (4) with 24° applied somewhat distally towards nearest cold spot for 9·7 sec. The temperatures were: surface 32·01°, 29·50° and 31·61°; 0·9 mm. 32·20°, 30·65° and 31·87°; 2·0 mm. 32·13°, 31·83° and 31·92°. Final record 36 sec. after end of stimulation. Sensation of cold during application with latency of 0·7 sec. and after-sensation of faint coolness.

15·40. Stimulation (5) with 23·5° for 6 sec., applied somewhat distally. The temperatures were: surface 32·48°, 30·46° and 32·41°; 0·9 mm. 32·72°, 30·65° and 32·63°; 2·0 mm. 32·75°, 32·58° and 32·75°. Final record 29 sec. after end of stimulation. Sensation cool; latency 1·5 sec.

15·43. Stimulation (6) with 23·5° for 8·1 sec., applied a trace distally. The temperatures were: surface 32·45°, 29·64° and 32·19°; 0·9 mm. 32·72°, 31·19° and 32·56°; 2·0 mm. 32·75°, 32·52° and 30·65°. Final record 30 sec. after end of stimulation. Sensation cool; latency 1·3 sec.

15·49. Stimulation (7) with 41·5° for about 7 sec. Records off scale. Sensation warm (approximate latency 1·3 sec.).

15·53. Stimulation (8) with 42° to 43° for 6 sec. The temperatures were: surface 32·69°, 35·54° and 32·78°; 0·9 mm. 32·96°, 33·98° and 33·03°; 2·0 mm. 33·03°, 33·23° and 33·03°. Final record 36 sec. after end of stimulation. No sensation except one of doubtful warmth some seconds after removal.

16·00. A number of punctures were made through drops of histamine (1 in 300 of ergamine phosphate); the punctures surrounded the warm spot at distances of 5 to 10 mm.

16·03. Area definitely hyperæmic. Stimulation (9) with 43° for 9 sec. a trace to one side of centre of spot. The temperatures were: surface 34·03°, >37·2° and 34·27°; 0·9 mm. 34·42°, 35·93° and 34·72°; 2·0 mm. 34·40°, 34·79° and 34·61°. Final record 32 sec. after end of stimulation. Sensation of considerable warmth; latency 2·7 sec.

16·05. Stimulation (10) with 43·5° with greater than average pressure; latency of sensation 1·2 sec. (see Fig. 3).

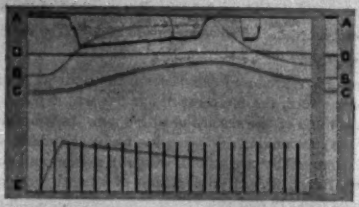

Fig. 3. Exp. 1, stimulation 10 (reduced ⅔). Stimulus of 43·5° for 11·3 sec., after induction of hyperæmia. The temperatures were: surface 34·4°, 37·95° and 34·65°; 0·9 mm. 34·65°, 36·55° and 34·95°; 2·0 mm. 34·6°, 35·6° and 34·9°. Sensation of "warmth," with latency of 1·2 sec.—becoming "hot" about 1 sec. later. After-sensation of warmth noted. An interval of 23 sec. is omitted.

16·19. Stimulation (11) with 24° for 17 sec. The temperatures were: surface 34·48°, 30·41° and 34·37°; 0·9 mm. 34·90°, 32·69° and 34·72°; 2·0 mm. 35·00°, 34·45° and 34·84°. Final records 24 sec. after end of stimulation. Sensation cool with pressure; latency 2·4 sec. The temperature sensation disappeared 5 sec. before stimulus was removed.

16·23. Stimulation (12) with 24·8° for 8·7 sec. The temperatures were: surface 34·67°, 31·72° and 34·44°; 0·9 mm. 35·05°, 33·69° and 34·85°; 2·0 mm. 35·13°, 34·84° and 34·97°. Final record 23 sec. after end of stimulation. Sensation of touch only.

16·25. Stimulation (13) with 29·8° (see Fig. 4).

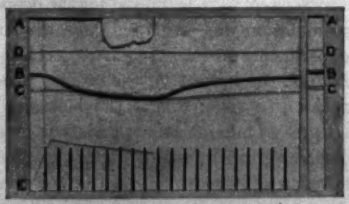

Fig. 4. Exp. 1, stimulation 13 (reduced ⅔). Stimulus of 29·8° for 8·4 sec. The temperatures were: surface 34·75°, 33·35° and 34·75°; 0·9 mm. 34·75°, 34·5° and 35·05°; 2·0 mm. 35·2°, 35·0° and 35·15°. Delayed, faint, but definite paradoxical sensation of warmth. An interval of 40 sec. is omitted.

16·29. Stimulation (14) with 25°. Records off scale.

16·32. Stimulation (15) with 24·5° for 7·6 sec. The temperatures were: surface 34·66°, 31·21° and 34·60°; 0·9 mm. 35·02°, 33·41° and 34·83°; 2·0 mm. 35·12°, 34·73° and 34·97°. Final records 22 sec. after end of stimulation. Sensation of faint coolness with a latency of 5·5 sec.; sensation lasted more than 1 sec. after removal of stimulus.

16·37. Stimulation (16) with 42·2° for 10·1 sec. The temperatures were: surface 34·71°, 37·44° and 34·98°; 0·9 mm. 34·98°, 36·31° and 35·13°; 2·0 mm. 35·05°, 35·28° and 35·18°. Final records 26 sec. after end of stimulation. An interrupted sensation of warmth with a latency of 4·0 sec. was noted during stimulation. A brief after-sensation of warmth was also recorded about 1·5 sec. after removal of the stimulus.

16·45. Thermocouples removed. Urine temperature 36·95°. The room temperatures were then at the higher values recorded.

The values and records reproduced demonstrate the complex gradients set up. After a warm stimulus, for instance, the temperatures at 0·9 mm. depth might be temporarily definitely greater than those of the surface or at 2·0 mm. Inflammatory hyperæmia rendered stimulation of the surrounding cold spots less frequent and less definite; paradoxical sensations of warmth from mildly cool stimuli were still present and possibly more marked (compare stimulations 1 and 13). After-sensations of warmth were only noted after the induction of hyperæmia (nos. 12 and 16).

Experiments with an applicator of 11 mm. diameter.

A series of experiments (2, 3 and 4) has been made on one subject (M.) in the winter, but in room conditions which were fairly close to a neutral level (17° effective temperature). A single experiment (5), complicated by some inflammation, has been made on subject B. In these experiments three loops were sunk at different depths and simultaneous records from these and a heavy surface thermocouple were obtained. The couples were located below a warm spot; several cold spots were within the area stimulated by the applicator. A torsion string galvanometer with a heavy thermocouple was used to record surface temperatures and D'Arsonval galvanometers were employed for the deeper thermocouples. The duration of stimulation was estimated from the surface record. In two of the three experiments on M., inflammatory changes were minimal.

Latency of sensation. In subject M. the latency of sensation showed considerable variation. To some extent the inconsistencies may have depended on the degree of inflammation; mainly it must have been due to other factors, such as errors in estimating the moment of application, variations in the pressure of the æsthesiometer, and the reaction time of the subject. Of these errors the last acts only in one direction tending to make the mean value greater than the true latency.

As a general rule the values observed tend to group about a median value. The minimum, maximum, arithmetic mean and the median values

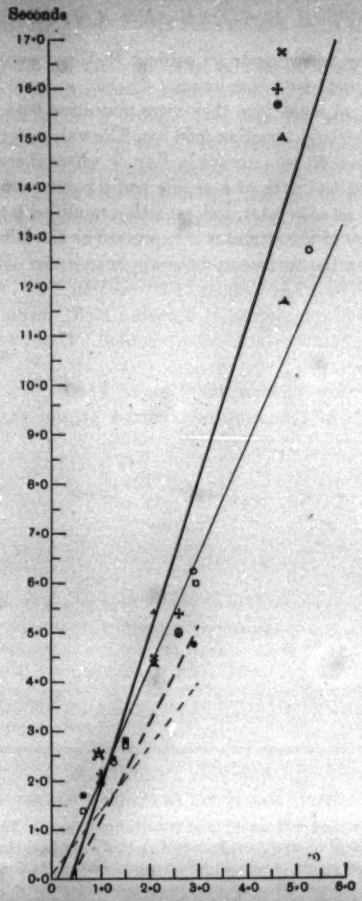

Fig. 5. The times in seconds, at which temperature changes could first be detected at different depths, are plotted as ordinates, the depths in mm. as abscissæ for data obtained in Exps. 2, 3, 4 and 5. Average times for Exp. 2 are represented by + for cold stimuli and ⊕ for warm; similarly, those for Exp. 3 by ○ and ●; Exp. 4 by △ and ▲; and Exp. 5 by □ and ■. Data obtained in Exp. 4 during stasis (warmth only) are represented by ×. The heavy continuous line represents approximately the rate of penetration of cold in subject M. only (Exps. 2, 3 and 4), without stasis, with an applicator of 11 mm. diameter; the lighter continuous line similarly that of warmth. The interrupted lines represent the penetration of cold (heavy line) and of warmth with an applicator of 1·5 mm. diameter in a warm environment.

are stated. In the experiments on subject M. these were for cold sensations: minimum 0·41 sec., maximum 1·16 sec., mean 0·70 sec., median 0·62 sec. For warm sensations they were minimum 0·45 sec., maximum 1·23 sec., mean 0·90 sec., median 0·98 sec. The values for cold were consistently less than 0·60 sec., except in Exp. 2, where there had been some hæmorrhage along the path of a needle and a consequent inflammatory reaction. As will be seen later, inflammation modified latencies of sensation. The intensity of the stimulus is expressed as the initial temperature difference between the surface and the applicator; for cold it was: minimum 12·0°, maximum 20·2°, mean 14·2°, median 14·0°. For warmth it was: minimum 9·5°, maximum 11·6°, mean 10·3°, median 10·0°. After-sensations of temperature were also recorded. These will be discussed later.

Rate of penetration of temperature changes. The times at which the first detectable change of temperature occurred at the various depths in

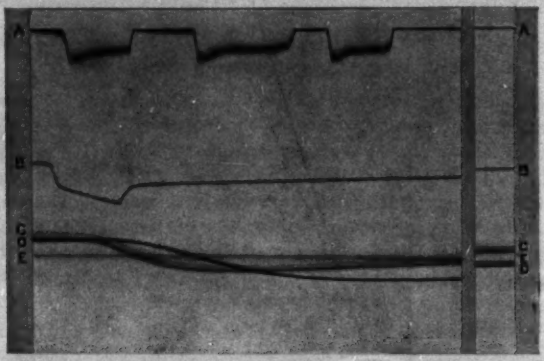

Fig. 6. Exp. 4. Warm and cold spots. Cold stimulus (reduced ½). The room conditions were: dry bulb 20·2° to 22·5°, wet bulb 9·6° to 12·8°, dry kata-thermometer 5·6 and 4·1 millicalories per sq. cm. per second, effective temperature (basic scale) 15·0° to 17·8°. In the records *A* represents sensations of temperature (depression); *B* surface changes with heavy thermocouple and torsion string galvanometer; *C* 0·95 mm. depth; *D* 2·1 mm.; and *E* 4·8 mm. Paper movement 4·5 mm. per second in Figs. 6, 7 and 8. Parallax corrections should be made by moving in the original records *A*, *C*, *D* and *E*, 0·2, 0·3, 0·5 and 0·4 mm. respectively to the left. In this experiment the stimulus was 18·4° applied for 5·4 sec. The initial, maximal and final temperatures were: surface 30·4°, 26·1° and 29·7°; 0·95 mm. 30·5°, 28·4° and 29·7°; 2·1 mm. 31·3°, 30·2° and 30·6°; 4·8 mm. 31·7°, 31·55° (also final). The sensations were primary "cold" and two after-sensations "slightly cool." An interval of 31·5 sec. is omitted.

Exps. 2, 3, 4 and 5 are shown in Fig. 5. The data were treated by the method of least squares and the straight lines in Fig. 5 were plotted from the resulting equations. A straight line does not represent the rate of penetration accurately; it gives a fair approximation considering the complexity of anatomical, physiological and physical factors. The progressive development of the temperature changes at the various depths may be seen in the actual records produced in Figs. 6 and 7. Attention

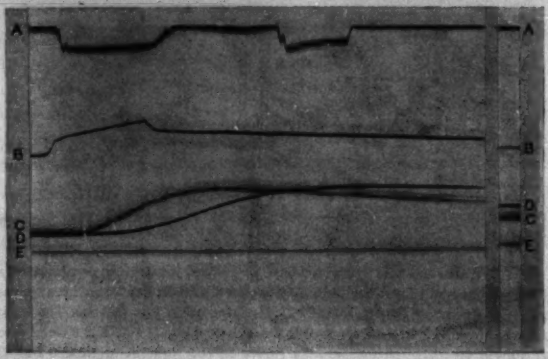

Fig. 7. Exp. 4. Warm stimulus (reduced $\frac{1}{2}$). Stimulus 40·8° applied for 7·5 sec. The initial, maximal and final temperatures were: surface 30·6°, 34·3° and 31·4°; 0·95 mm. 30·7°, 33·5° and 31·6°; 2·1 mm. 31·3°, 32·6° and 32·1°; 4·8 mm. 31·8°, 31·9° (also final). The sensations were a primary "warm changing to hot" (duration indicated by the first depression of record *A*) and an after-sensation "slight warmth." An interval of 38 sec. is omitted.

should be directed to the late onset, slow development, and long persistence of the temperature changes at the greater depths.

In Fig. 5 average values of two tests of the rate of penetration of warmth after production of stasis are also plotted. Stasis was produced by a Riva Rocci armlet at the level of the elbow maintained at 200 mm. Hg pressure. Records were obtained 8 and 15 min. after the start of compression. Stasis produced only a slight slowing of penetration, except at the greatest depth; the rate of penetration is less accurately represented by a straight line; the amount of change was actually increased, but the temperatures reached were not as great, for the initial levels were lower; the maximum temperatures were reached somewhat early. Stasis produced no profound change in the type of record obtained.

Complicated curves. In a small proportion of the records complicated curves may be obtained in which the recorded temperature changes may temporarily be opposite in direction to those anticipated (Exp. 5, Fig. 8).

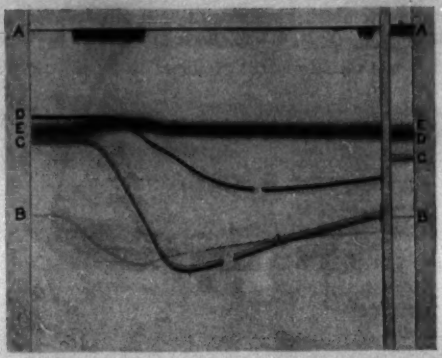

Fig. 8. Exp. 5. Warm and cold spots (reduced ½). Record illustrating complicated reverse curves. Room conditions: dry bulb 27·1°, wet bulb 20·8°, dry kata-thermometer 3·1°, effective temperature (basic scale) 22·7°. Inflammation was present. The indices are similar to those of Fig. 6: *C* represents a depth of 0·65; *D* 1·5; *E* 2·9 mm. Record *A* only should be corrected for parallax (move to the right in original 0·1 mm.). The stimulus was 18·8°, applied for 5·3 sec.; applicator 11 mm. diameter. The initial, maximal and final temperatures were: surface 35·7°, 26·4° and 34·8°; 0·65 mm. 36·4°, 31·7° and 35·4°; 1·5 mm. 36·4°, 33·85°; 2·9 mm. 35·55°, 35·3° (also final), and, 4·5 sec. after initiation of stimulation, an apparent increase to 35·8°. The primary sensation was one of cold, followed by three after-sensations (one omitted) of the same character. An interval of 33 sec. is omitted.

A rise of temperature with a cold stimulus, as occurred in this experiment at a depth of 2·9 mm., was never seen in superficial couples. A step-like fall, as was recorded by the thermocouples at 1·5 mm. and to a less extent at 0·65 mm. depth, was not infrequent, and was always more marked the deeper the position of the thermocouple, as in this case. Curves of this type were always associated with definite pressure on application, and the reversal and steps in the curve were related to the cessation of application. It may also be noted that when a temperature of the reverse direction is recorded (*e.g.* at 2·9 mm.) this change occurs immediately the applicator is applied without any lag such as is normally associated with conduction of temperature changes. A possible explanation is that a thermocouple may by chance lie between an artery and a vein and being

affected by both record a value intermediate between their temperatures; if an applicator be applied in such a manner as to interfere with the blood flow in either vessel, the temperature of the thermocouple would vary. These reversed temperatures are not recordable at depths at which the end-organs for temperature probably occur; they are probably not related to paradoxical sensations of temperature.

Experiments with an applicator of 1·5 mm. diameter.

A total of 41 experiments has been made with an applicator of 1·5 mm. diameter. Owing to their number the data are treated collectively. It has been assumed that the individual differences of the three subjects mainly employed were of less significance than chance errors. All the data were obtained with thermocouples of the lightest wire and D'Arsonval galvanometers, except for some observations on latency of sensation obtained in earlier experiments with needle thermocouples.

Latency of sensation. The values obtained exhibit considerable inconsistencies. In addition to the errors possible with a larger applicator, the manipulator of the æsthesiometer might make the application lateral to the sensitive spot, thus delaying its temperature change and increasing the latency. The inconsistencies were, however, due in part to the temperature conditions of the room, since they are much less in evidence if the experimental results are grouped according to environmental conditions.

The data are presented in Table I. In a cold environment, where the

Table I. Latency of sensations.

			Stimulus		Latency of sensation			
Sensation	Condition	No. of observations	(range ° C.)	Median (° C.)	Min. (sec.)	Max. (sec.)	Mean (sec.)	Median (sec.)
Cold	Cold room	10	− 7·4 to −13·0	−10·3	0·1	1·0	0·37	0·30
	Warm room (needle couples)	33	− 6·0 to −23·5	−11·0	0·0	2·0	0·78	0·60
	Warm room (loop couples)	9	− 8·1 to −12·2	− 8·5	0·8	3·5	1·64	1·60
	Hyperæmia	5	− 9·1 to −21·8	−11·4	0·3	5·8	2·52	1·2
Warm	Cold room	7	+12·7 to +15·9	+14·0	0·6	2·8	1·11	0·9
	Warm room	20	+ 4·4 to +12·5	+7·15	0·0	2·4	1·25	1·25
	Hyperæmia	11	+ 6·9 to +11·5	+ 9·3	0·8	4·0	1·45	1·2

data were few, they were for the most part consistent; the latencies were not affected by the type of thermocouple inserted. However, in a warm environment the data, though more numerous, vary considerably and show a striking contrast in the latency of cold sensations according to

the type of thermocouple employed. The needles usually lay at a considerable depth below the probable position of the end-organs; the track of the loops must often have been less than 1 mm. distant from them. The contrast may be due to inflammatory changes which during the preliminary period were more noticeable in a warm environment. To test the effect of inflammation a few data were obtained after the induction of hyperæmia by histamine punctures or scalds 5 or more mm. distant; in these experiments the room conditions were usually cool (13·3° to 18·8°, mean 16·1° effective temperature). The data so obtained showed much greater variations than under normal conditions, even though all data were discarded where recognizable errors were present.

Comparison of latencies. If the few data obtained with loops (in the probable presence of inflammation) with a cold stimulus be excluded, the latency of cold is consistently much shorter than that of warmth. The data also clearly demonstrate a longer and more irregular latency for both warm and cold sensations in a warm room and in the presence of hyperæmia induced by inflammation. The variations in latency with room conditions cannot be accounted for by variations in the strength of the stimulus, for responses to both cold and warmth were delayed in warm rooms, although the stimulus for cold was actually increased. The effect would appear to be due to the physical condition of the skin, since alterations may be observed also in the conduction of temperature changes.

After-sensations. After-sensations of temperature, occurring after removal of the stimulus, have been a common occurrence in these experiments, even though often only a single sensitive spot could be identified in the area stimulated. Those of cold have been much more definite and consistent than those of warmth; they have persisted for a long period and often have faded and returned several times. They have sometimes been so definite that an inexperienced subject has thought the applicator has been applied more than once. They have occurred at a time when the surface temperature was returning towards its previous level, and when the temperature changes at the deepest levels produced by the stimulation were still developing. Examples may be seen in Figs. 3, 6, 7 and 8.

Rate of penetration of temperature changes.

Temperature changes on the surface. When a light thermocouple was placed on the surface, and the temperature changes were recorded at the time of the stimulation, the lag of the galvanometer system did not prevent the detection of the moment of contact determined simultaneously by an electrical signal.

The character of the temperature changes might be expected to resemble those of the cooling body and to be expressed by an exponential equation; this was approximately true. If θ_0 represents the initial difference in temperature between the skin and the applicator, and θ_t the difference between the temperature at the skin-applicator boundary (recorded by the thermocouple) and the temperature of the water in the æsthesiometer after application for time t, then, for periods up to 5 or 6 sec., the temperature change is represented by the formula:

$$\theta_t = \theta_0 e^{-at},$$

where a has a value of 0·04 to 0·06. The equation does not hold for longer periods, since it supposes that the surface will assume ultimately the temperature of the applicator, and, owing to the transference of heat by the circulation, this never occurs. Consequently, when the data are plotted semi-logarithmically, the line is slightly curved, and the average value for the constant is smaller the longer the duration of stimulation. In addition, chance variations occur, probably mainly due to variations in the angle or pressure of application.

A series of data on cold stimulation (Table II) renders the degree of variation apparent. The mean value of the constant is 0·046, with a stimulus of an average duration of 8·09 sec. The vascularity of the skin does not appear to make much difference, since in experiments with a cold stimulus during inflammatory hyperæmia the mean value of the constant is 0·05 for a stimulus of average duration of 9·85 sec. When a warm stimulus is used the changes are similar, but the mean value of the constant is somewhat greater; for a stimulus of average duration of 7·56 sec. its mean value is 0·065; with inflammatory hyperæmia and stimuli of average duration of 8·24 sec. its mean value is 0·063. A considerable period of time is required for the skin surface even to approximate to the temperature of the water in the applicator. Thus by calculation with a warm stimulus in the presence of hyperæmia the difference in temperature between the skin and the applicator should be reduced from 10·5° to 4·5° in 17 sec.; by observation the difference was 6·4°; the discrepancy was probably due to the gradual alteration in slope.

On removal of the stimulus the surface temperature returned towards its previous value with an exponential type of curve. Again, no exact curve could be calculated from the difference between the temperature at the end of stimulation and a final temperature, estimated on the basis of the initial temperature. When plotted semi-logarithmically a slight curve is obtained, suggesting that a temporary equilibrium may be

TABLE II. Change of surface temperature with cold stimuli and applicator of 1·5 mm. diameter.

The experiments are listed in order according to effective temperature of the environment, commencing with cold conditions. The applications at a temperature of 29° to 32° did not g ve any sensation of cold.

No. of exp.	Initial surface temp. (° C.)	Applicator temp. (° C.)	Final surface temp. (° C.)	Duration of stimulus (sec.)	a
6	30·25	20·2	27·9	6·1	0·044
7	30·9	21·6	27·45	6·9	0·067
8	29·2	21·8	26·65	11·5	0·037
	29·05	20·8	27·0	7·9	0·037
9	33·15	25·0	32·3	4·9	0·023
	33·15	25·0	31·0	6·3	0·049
	34·35	25·0	31·6	12·6	0·030
1	31·1	29·6	30·7	8·6	0·039
	32·0	24·0	29·5	9·3	0·042
	32·5	23·5	30·5	6·0	0·042
	32·5	23·5	29·65	8·1	0·046
10	32·15	20·2	29·75	9·4	0·028
	32·0	19·8	28·25	8·6	0·044
	32·4	29·8	31·5	10·2	0·042
11	33·0	24·8	30·1	6·0	0·074
	32·85	31·6	31·95	12·7	0·100
12	33·65	31·0	32·65	7·7	0·061
13	33·0	31·0	32·65	8·3	0·023
	32·85	30·6	32·15	6·9	0·056
14	33·3	24·0	30·55	7·3	0·048
	33·4	24·0	30·5	6·8	0·055
	33·6	32·0	33·3	5·8	0·036
			Average values	8·09	0·046

reached somewhat below the previous level after cold stimuli, and above it after warm. The previous temperature was often regained in 3 or 4 minutes, but would then swing slightly above this level after a cold stimulus, or below it after a warm. The over-swing was particularly in evidence in subject M., while, on the contrary, in subject B. there might be no return to the initial temperature even after an interval of 5 minutes. The results are most readily explained on an assumption of temporary vaso-constriction and vaso-dilatation.

Temperature changes deep to the surface. The latencies of the temperature changes are definite and show an approximate linear relationship to the depth. Data obtained with both cold and warm stimuli in a cold environment are plotted in Fig. 9; the curves drawn represent the best-fitting straight lines determined by the method of least squares after grouping the data according to $\frac{1}{4}$ mm. variations in depth. Lines similarly determined (on more numerous data) for a warm environment are

shown in Fig. 5 (interrupted lines). The positions and slopes of these lines clearly vary with the character of the stimulus and the environmental

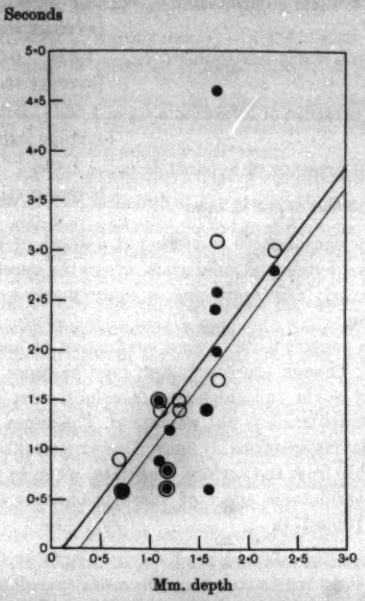

Fig. 9. Rate of penetration of cold and warmth in a cold environment. The depths in mm. are plotted as abscissæ, the times in seconds at which temperature changes could be first detected as ordinates. Data obtained with the lightest loop thermocouples, D'Arsonval galvanometers and an applicator of 1·5 mm. diameter. Individual data obtained with cold stimuli are plotted as open circles, those with warm stimuli as solid circles. The heavy line represents the rate of penetration of cold, the lighter that of warmth.

condition; the variations probably depend on the initial vascular condition and on vascular readjustments during stimulation. Inflammatory hyperæmia also modifies the rate of penetration. The lines of Figs. 5 and 9 (1·5 mm. data only) and the rate of penetration in the presence of inflammation may be represented by the following equations:

Cold stimulus in cold environment $t_{c_c} = 1{\cdot}32d - 0{\cdot}16$

(average stimulus — 8·9°);

Warm stimulus in a cold environment $t_{W_c} = 1{\cdot}32d - 0{\cdot}36$ (average stimulus + 13·7°);

Cold stimulus in a warm environment $t_{C_c} = 1{\cdot}91d - 0{\cdot}63$ (average stimulus − 8·9°);

Warm stimulus in a warm environment $t_{W_w} = 1{\cdot}33d + 0{\cdot}06$ (average stimulus + 8·1°);

Cold stimulus in presence of hyperæmia $t_{C_h} = 1{\cdot}66d - 0{\cdot}53$ (average stimulus − 11·5°);

Warm stimulus in presence of hyperæmia $t_{W_h} = 1{\cdot}38d$ (average stimulus + 9·7°);

where d represents the depth in mm.

The data show considerable scattering at a depth of 1·5 to 2·0 mm., the junction of the dermis and subdermis, where the vascular conditions may be complicated; this scattering is more marked when there is vascular dilatation.

The equations suggest a very rapid conduction of heat in the most superficial layers. Though the conduction may be more rapid in these than in deeper areas, the equations are not valid for depths of less than 0·5 mm. since extrapolation is not warranted. The times of penetration in the superficial layers are probably more closely represented by assuming a linear relationship from the surface. The data, when so presented, still demonstrate a considerable effect of vascular changes on the rate of penetration (see Table III).

TABLE III. Mean velocity of conduction in mm. per sec. up to depths of 1 mm. (1) estimated from data at 0·6 to 0·7 mm. depth, (2) from data at about 0·9 mm. depth.

	Cold stimulus		Warm stimulus	
	(1)	(2)	(1)	(2)
Cold environment	0·99	—	1·22	—
Hyperæmia	1·03	0·82	0·59	0·72
Warm environment	0·77	0·76	0·77	0·86

Vascular dilatation, whether produced by inflammation or by a warm environment, slows the rate of penetration for both warmth and cold (with the possible exception of cold in the most superficial layers). Warmth appears to be conducted more rapidly than cold except in the most superficial layers in the presence of vascular dilatation where it is conducted more slowly.

When the temperature change has once become detectable, it develops rapidly its maximal rate of change. When the intervals between application and the attainment of the maximal rate of change (t') are

plotted against depth, the best fitting lines give the following equations:

Cold and warmth in cold room, $t' = 3{\cdot}0d - 0{\cdot}3$.

Cold and warmth in warm room, $t' = 2{\cdot}33d + 0{\cdot}55$.

Cold and warmth in presence of hyperæmia, $t' = 2{\cdot}24d + 0{\cdot}55$.

These equations only indicate the approximate times recorded for depths up to 2 or 3 mm.; the actual interval must be less, as in this case instrumental lag produces a considerable error.

The rise and fall of temperature during stimulation follow approximate exponential curves, although at the greater depths definite diphasic curves are often recorded, and changes in blood flow complicate the issue. The moment at which the maximum temperature change is reached is determined by the duration of the stimulus, but lags behind it to a degree dependent on the depth. The amount of the maximum change of temperature varied with the temperature difference between the skin and the applicator and the duration of the stimulus; it varied inversely with the depth. It is impossible to include these factors in an equation, since the change also varied considerably with the pressure and angle of application, and the consequent effects on the circulation.

The threshold of stimulation. When a stimulus at a temperature close to that of the surface is applied, temperature changes are recorded by the thermocouples which are not associated with stimulation of the end-organ investigated. The subject may report a tactile sensation only, or may claim to have experienced a faint temperature sensation opposite in character to the direction of the temperature change (see Fig. 8). Apparently the thermocouples can record temperature changes below the threshold for a single end-organ.

Comparison of temperature changes produced by applicators of different size. The change in temperature on the surface or at a depth below it is greater the larger the diameter of the applicator. A few data have been grouped for comparison in Table IV. The data of Fig. 1 (Table IV, no. 9) were obtained under conditions similar to those which gave a value of 0·065 for the constant a (see p. 95); with the larger applicator this value becomes 0·17.

Discussion.

Temperature changes and size of applicator. In previous work a tacit assumption has usually been made that the temperature on the surface during stimulation is identical with that of the applicator. The data presented demonstrate that this is not the case, that the temperature of the

applicator is only slowly approached and never reached. It has long been recognized that the threshold stimulus below which temperature changes are not recognized varies considerably with the area stimulated. Though to a large extent these variations may depend on spatial summation of subliminal stimuli, yet they must depend partly on the magnitude of the temperature changes, since it can be demonstrated that both the rate and magnitude of temperature change at any point, whether on the surface or deep to it, are also greater the larger the surface of the application (Table IV).

TABLE IV.

No.	Stimulus applicator diam. (mm.)	Temp. (° C.)	Duration (sec.)	Initial temp. (° C.)	Temp. at maximal change (° C.)	Depth of 0·9 to 1·0 mm. Initial temp. (° C.)	Depth of 0·9 to 1·0 mm. Maximal change (° C.)	Remarks
1	11·0	16·8	6·2	30·55	25·7	30·95	28·95	Exp. 2
2	1·5	16·5	5·6	30·75	27·45	31·05	30·65	,,
3	11·0	17·3	8·7	30·95	25·75	30·95	28·70	,,
4	1·5	17·3	6·3	30·3	27·3	30·85	30·4	,,
5	11·0	41·0	5·4	31·2	36·0	31·15	33·5	,,
6	1·5	41·0	5·9	31·6	34·45	31·35	31·85	,,
7	11·0	19·2	5·2	32·9	24·91	—	—	
8	1·5	19·8	8·6	32·0	28·25	32·55	31·75	Table II, Exp. 10
9	11·0	41·5	5·4	33·6	38·25	33·97	35·92	Fig. 1
10	1·5	42·5	6·0	32·7	35·55	32·95	34·0	Exp. 1 (8)
11	11·0	41·1	5·7	30·8	34·5	30·9	32·8	Exp. 4

Vascular factors altering rate of penetration. When the blood flow is increased, both the thermal capacity and conductivity of the tissues must be greater, and in regard to the deeper tissues these changes should act in opposite directions and appear to be more or less balanced. The differences observed under varying environmental conditions are explicable by this balance being incomplete if one assumes that local vaso-constriction and dilatation are also induced by the stimuli themselves. In spite of the greater temperature changes with a large applicator, the rate of penetration is slower; probably this is caused by interference with blood flow through the compression necessary to make the applicator lie flat. This compression might at first empty the capillaries into the venules and temporarily quicken the flow in the most superficial layers.

The rate of penetration is very much more rapid than that postulated by Goldscheider(7) and Pütter(11). Pütter calculates that the maximum rate of change should not be reached at 1 mm. depth for about 100 sec.; it is recorded after an interval of only 2·7 sec. (1·5 mm. applicator) or of 5·5 sec. (11 mm. applicator) and must actually occur somewhat earlier than that. The time required does not appear to vary with

the square of the depth, as demanded by their equations, which cannot be properly applied to vascular tissues.

Vascular factors affecting sensation. The latencies for sensations of both cold and warmth are shown to be shorter in a cold environment than in a warm one, and these variations in latency appear to be paralleled by and probably dependent upon variations in the rate of penetration of the physical changes.

Inflammation was also found to decrease the sensitivity to cold stimuli, and to increase the latency; on the other hand, it appeared to increase the sensitivity to warm stimuli, though increasing the latency (to a less extent) in this case also. After the introduction of loop thermocouples no paradoxical sensations of pure cold could be elicited by a hot stimulus, although they had been previously observed.

The profound effect of inflammation on cold sensations does not appear explicable on the basis of altered rate of penetration alone; apparently a condition of capillary constriction, either initial or induced, is essential for the ready production of this sensation. Before the introduction of the thermocouples paradoxical sensations of warmth produced by cool stimuli were never noted; after the introduction they were not infrequent, might be quite definite, and were usually exaggerated by histamine hyperæmia. The existence of such paradoxical sensations has given rise to much controversy (Ebbecke(4), Goldscheider and Hahn(8)); mechanical stimulation has often been considered the main factor. Since vascular dilatation from inflammation interferes with the elicitation of paradoxical cold, the fact that Goldscheider(7) only noted paradoxical sensations of warmth with stimuli above 22° C. might depend on the prevention of this sensation by vaso-constriction induced by extreme cold. The facilitation of these sensations by inflammation might also be explained by vascular changes rather than by increased mechanical excitability of the end-organs.

After-sensations. When only a single sensitive spot was stimulated, definite after-sensations were reported, contrary to the observations of others; but in these experiments applicators of considerable diameter were employed, since a simple sharp pointed cone could not be made to cover both the spot and the thermocouple. The larger surface would result in transference of much larger quantities of heat than would occur with a smaller area.

The after-sensations reported might depend on the later development of temperature changes either at deeper levels or in areas situated laterally to the end-organs investigated. Such an hypothesis in its simplest

form seems untenable, since after-sensations of cold were never recorded when cold spots were absent from the spot primarily stimulated, even though cold spots might exist at only a slight distance lateral to the point of application. Since, however, the rates of penetration of heat and cold are different, a local vasomotor response to the stimulus appears to accompany primary sensations of cold and this might be an essential factor in the stimulation of laterally situated end-organs with an application involving an area of only 1·8 mm.^{2}. In this way the results here reported might be brought into agreement with the data on after-sensations reported by v. Frey(6). If the sensations arise in the end-organs originally stimulated, they develop when the temperature of the whole area is rising, but if they arise in other end-organs, laterally situated, as the result of peripheral spread and vaso-constriction the results would, like those of v. Frey, be explicable on Weber's hypothesis.

Calculated depth of end-organs. The depth of the end-organ may be calculated approximately by relating the latency of sensation to that of thermal penetration, after allowing for the time taken in nervous processes (on the basis of other sensations this time has been assumed to be about 0·15 sec.). If a linear rate of penetration of thermal changes up to 1 mm. is assumed, and if the time taken for the temperature change to reach its threshold value is neglected, maximal depths may be calculated, since a measurable time is probably taken to reach the threshold change.

On this basis the depth of the end-organ for cold is calculated to be 0·4 mm. (Exp. 2 with inflammation), 0·2 mm. (Exp. 3), 0·15 mm. (Exp. 4) from data obtained with an applicator of 11 mm. diameter. From the data obtained in a cold environment with an applicator of 1·5 mm. diameter a depth of 0·15 mm. is derived. Inflammation as the result of the thermocouple introduction was much more marked in a warm environment and interferes with the use of these values.

Neglecting all data where inflammation has probably played a rôle, a depth for this organ of 0·15 mm. or less may be assumed. This would correspond with an end-bulb of the Krause type, if present in the papillæ (Strughold and Karbe(12)) or less probably with end-organs in the Malpighian layer (Pendleton(10)). The discrepancies between physiological data and histological descriptions are discussed by Pendleton.

The depth of the end-organ for warmth may be calculated in a similar manner. The depths obtained are: 0·4 mm. (Exp. 2), 0·5 mm. (Exp. 3), 0·35 mm. (Exp. 4), 0·8 mm. for the experiment illustrated in Fig. 1, when an applicator of 11 mm. diameter is employed; with an applicator of

1·5 mm. diameter the values so calculated are 0·9 mm. in a cold environment, 0·8 mm. in a warm environment and 0·6 mm. in the presence of inflammatory hyperæmia.

Since inflammation does not appear to produce any specific effect on sensations of warmth, the threshold stimulus need not be assumed to be infinitesimal. Calculations may be made on the assumption that the latency of sensation has three factors; one with a value n representing nervous processes; another d/v representing the time taken for the temperature changes to penetrate to depth d, when conducted at v mm. per sec.; and a third x representing the time taken to reach the threshold stimulus. To estimate this third value consideration has to be given to the rate at which the temperature alters.

An estimate of the differences in the rate of growth has been made by plotting the results of some experiments semi-logarithmetically and calculating the slopes of the smoothed curves (based on the first 6 sec.). Values of the constant a for equations of a type similar to that employed for changes in surface temperature were calculated; for depths of 0·9 mm. these were with an applicator of 11 mm. diameter 0·035 (Exp. of Fig. 1) and 0·039 (Exp. 4); with an applicator of 1·5 mm. diameter in a warm room 0·0215 (Exp. 1, stimulus 8, before hyperæmia was induced) and 0·0285 (Exp. 1, stimulus 16, after induction of hyperæmia). These figures demonstrate not only a great difference between the applicators of different size, but also the effect of hyperæmia. The latter effect is much greater if comparison is made with the curves obtained in a cold environment. Analysis of curves obtained at 1·1 mm. depth in Exp. 8 gave a mean value of 0·0155 (three experiments) before hyperæmia, and analysis of a single curve after induction of hyperæmia in the same experiment gave one of 0·0225. Apparently the rate of growth of temperature changes is a function not only of the strength of the stimulus (temperature difference and area of applicator), but also of the vascular condition of the skin. If the conditions in a cold environment with a 1·5 mm. applicator and stimulus of + 14·0° be taken as the standard of reference, the ratios for the times taken for equivalent changes in temperature in the first second (determined from values of θ_0 and a) for the other conditions are approximately: warm environment 1·43; hyperæmia 0·92; 11 mm. applicator, in a warm environment (Fig. 1) 0·77; in a neutral environment with pressure (Exp. 4) 0·54. The probable time taken to reach the threshold value for any condition and stimulus may then be expressed in terms of the time taken in a cold environment with a stimulus of + 14·0°, this time being considered as x seconds. With an applicator

of 1·5 mm. diameter employing the median values for the latency of sensation (t_s) the following equations are obtained:

$$t_s = 0{\cdot}9 \text{ sec.} = n + \frac{d}{1{\cdot}22} + x \text{ (cold environment);}$$

$$t_s = 1{\cdot}25 \text{ sec.} = n + \frac{d}{0{\cdot}77} + 1{\cdot}43x \text{ (warm environment);}$$

$$t_s = 1{\cdot}20 \text{ sec.} = n + \frac{d}{0{\cdot}59} + 0{\cdot}915x \text{ (hyperæmia);}$$

and they give the following values

$$n = 0{\cdot}19 \text{ sec.}, \; d = 0{\cdot}39 \text{ mm.}, \; x = 0{\cdot}39 \text{ sec.}$$

The values so calculated must only be considered as an indication of the probable values, since many assumptions have been made, and the values for latency are themselves very variable. In fact, if the mean values are used instead of the medians, an absurd value for x is found

$$(n = 0{\cdot}69 \text{ sec.}, \; d = 0{\cdot}40 \text{ mm.}, \; x = -\,0{\cdot}10 \text{ sec.}),$$

demonstrating the danger of trusting too implicitly to such calculations, even though the value of d is little altered.

If allowance be made on this basis for the time taken to reach the threshold in the experiments with an 11 mm. applicator, the depths calculated are 0·3 mm. (Exp. 4) and about 0·55 mm. (Exp. of Fig. 1). In Exp. 4 a relatively heavy surface thermocouple of 0·2 mm. wire was employed which would be forced by compression into the tissues altering their relations. X-ray photographs demonstrated the possibility of the compression of the tissues and of the reduction of the depth of a thermocouple at 1 mm. depth by this means by some 0·2 to 0·3 mm. with pressures such as were employed in these experiments. Such compression might account for discrepancies, particularly if combined with irregular effects of compression on blood flow in superficial and deep vessels. The assumptions made that the lag of the galvanometers is negligible (when comparison is made with a second galvanometer record), and that the rate of thermal penetration can be represented by a straight line, probably both introduce errors slightly minimizing the calculated depth of the end-organ. The value of x calculated is somewhat large, implying a rise of 0·1° to 0·2° before stimulation of the single end-organ occurs; it may be somewhat above the true value (see François and Piéron(5)). The depth of the warm end-organ seems likely to be between 0·4 and 0·6 mm., but in any case within the dermis and not in the subdermal tissues as supposed by Ebbecke(4). On the other hand, it appears to be definitely deeper than that of cold and not at the same level as supposed by Goldscheider

and Hahn(8). Further work, which attempts to establish more definitely the structure and relationships of the end-organs concerned, is in progress.

We are indebted to Dr G. M. Endres for a personal communication of data obtained in an entirely different manner which suggest similar depths for the end-organs of cold and warmth. His experiments will be published soon in the *Zeitschrift für Biologie*.

Summary.

1. Light thermocouples have been introduced into the skin of the forearm in the immediate neighbourhood of warm and cold spots, and photographic records have been obtained of the temperature changes which accompany temperature sensations. The stimulus has been an æsthesiometer of either 1·5 or 11 mm. diameter.

2. Data are presented illustrating the magnitude of the temperature changes on the surface and within the dermis, with various stimuli, and with applicators of different size. The magnitude of the temperature change is demonstrated to be a function of the size of the applicator, and the strength of the stimulus. The skin during stimulation does not attain the temperature of the applicator as has been usually assumed.

3. The main thermal change penetrates the tissues with a velocity of 0·5 to 1·0 mm. per sec. and the rate varies with the vascular conditions. Vascular dilatation slows the rate of penetration (with the possible exception of cold in the most superficial layers). Warmth is conducted more rapidly than cold under all conditions except in the most superficial layers of the skin.

4. Latencies of sensation were measured under various environmental conditions and showed variations according to the vascular state. Latencies for both cold and warmth were longer in a warm room. Inflammation interfered with sensations of cold and exaggerated their latency; it also interfered with paradoxical sensations of cold.

5. By correlation of latencies of sensation with the rate of penetration estimates of the depths of the end-organs concerned have been made. These are: 0·15 ± 0·1 mm. for the end-organ for cold, and 0·6 ± 0·2 mm. for that for warmth. The possible errors and various methods of calculation are discussed. The end-organ for warmth cannot lie in the subdermal tissue (*i.e.* deeper than 1·5 to 2·0 mm. in the forearm).

6. Paradoxical sensations of warmth have been noted in response to cool stimuli and appeared facilitated by inflammation.

7. Some description is given of complicated curves which may be recorded at the greater depths and which sometimes have indicated temperature changes opposite in sign to those anticipated from the stimulus. These changes appear to be due to variations in blood distribution produced by the pressure of the applicator, and are not related to paradoxical sensations.

8. After-sensations of both cold and warmth were recorded; their relation to changes of temperature is discussed.

9. The data do not warrant discussion of the mode of stimulation of the end-organs at present; more exact information of the anatomical structures concerned is necessary.

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THE SOURCE OF CO_2 EXPIRED AND THE SITE OF ITS RETENTION.

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After evisceration the respiratory quotient (R.Q.) of a cat maintained by artificial ventilation and receiving glucose injections rises to about one (Burn and Dale). These experiments were repeated by Kilborn(2), using eviscerated cats without extra sugar, showing that the R.Q. rose to one or even above. But he showed that the eviscerated animals were being over-ventilated, and he considered that on that account there was no proof for a rise in true R.Q. In a previous paper(1) we have shown that in the eviscerated cat over-ventilation diminished the muscle CO_2 content as well as that of the blood, and that the observed loss in CO_2 from blood and muscle accounted for from 10 to 30 p.c. of the extra CO_2 calculated by Kilborn's method as that blown off by over-ventilation.

By demonstrating that the CO_2 reserve in the muscles contributed CO_2 during over-ventilation after evisceration, Kilborn's view that the rise in R.Q. did not represent a change in metabolism was sustained. The actual source of all of the extra CO_2 was not, however, determined. In examining the tissues for possible sources of the unexplained extra CO_2, it became apparent that only the bones contained a sufficient amount of CO_2 to supply the unexplained extra quantity expired, if it originated in a preformed source. But the bones were so rich a source of CO_2 that only very close analytical comparisons could have revealed the loss of the unexplained extra CO_2, and for this reason the results were not conclusive.

The problem has a general as well as particular interest when we wish to determine the extent of the carbonate reserves of the animal which are labile enough to respond rapidly to alterations in alveolar CO_2 tension. As the decapitated eviscerated cat is a rather variable preparation, other preparations have been selected which would be better fitted to indicate the extent and situation of the labile body carbonates.

The over-ventilated cat shows a similar adjustment to diminished

alveolar CO_2 tension in giving off extra CO_2. If the degree of over-ventilation is not great, no severe symptoms appear. Recovery is prompt, and there is no reason to suspect an alteration in the type of metabolism. The converse type of experiment is shown where cats are curarized and caused to breathe CO_2 rich mixtures. In these experiments Shaw (5) has shown that after a period of CO_2 retention a return to the original R.Q. is observed. The animal apparently adjusted itself to a state of higher CO_2 tension without any effect on the type of metabolism. In over-ventilated cats and those ventilated with CO_2 rich mixtures there is no reason for suspecting a change in the true R.Q. We have therefore repeated experiments with cats during over-ventilation and ventilation with CO_2 rich mixtures, examining (1) the expired air as an indicator of CO_2 lost or gained, and (2) blood, muscle and bone for changes in CO_2 content to explain the source of extra CO_2 expired, or site of its retention.

Experimental procedure.

For over-ventilation experiments cats under amytal anæsthesia were prepared with tracheal and carotid cannulæ. Normal samples of expired air were then taken by inserting a Pearce animal membrane valve between the tracheal cannula and the 5-litre rubber collecting bag. From the bag about 50 c.c. were at once transferred to a Brodie gas pipette and stored, under mercury seal, until analysis. A sample of arterial blood was allowed to flow directly into an Ostwald pipette and transferred to the Van Slyke apparatus. Muscles were then excised from one leg and kept in liquid air until analysed. Bones were removed from the same leg, bleeding was arrested, and the cat covered on the warmed table.

Over-ventilation was effected by a pump with sliding metal valves. The pump was shown to deliver accurately all the air taken in, and was adjustable as to volume per stroke and stroke per minute. The inspired air was metred. At about half-hour intervals 5-litre samples of expired air were collected and about 50 c.c. transferred to the Brodie gas pipettes. Immediately after taking the last air sample, arterial blood was drawn and the muscles and bones removed from the other leg.

Analytical procedure.

Expired air samples were analysed for CO_2 and O_2 in a series of Haldane gas analysis apparatus. From these analyses the CO_2 produced and O_2 consumed per 100 c.c. of expired air were calculated by comparison with inspired air corrected on the basis of nitrogen content. The total CO_2 produced and O_2 consumed for any period was obtained by

multiplying the p.c. CO_2 produced and p.c. O_2 used by the volume of air expired during the period. This in turn was calculated from the volume of inspired air. The arterial blood CO_2 contents were determined according to the methods of Van Slyke and Neill(6); CO_2 combining power of blood was determined (after equilibration at 38° C.) in the Van Slyke apparatus. Muscle CO_2 was also determined in a Van Slyke blood gas apparatus adapted for receiving solid pieces of muscle(3). The most suitable muscles were small ones which could be quickly dissected free from fascia and used whole or in definite corresponding parts, such as Extensor Digitorum, Peroneus Longus and Tibialis Anticus. Normal right and left muscles agree in CO_2 content to within 5 p.c. with an average percentage deviation from the mean of each pair of muscles of 1·4 p.c. For bone CO_2 determinations the whole fibula or part of a tibia was dissected out, cleaned and weighed. The CO_2 was expelled by acid and collected in weighed absorption tubes. The average discrepancy in CO_2 content shown between right and left corresponding bones was less than 2 p.c.

Results of over-ventilation.

Typical results of an experiment are listed as Exp. 1, and four experiments are summarized in Table I. These show a CO_2 loss during over-ventilation, assuming that no change in true R.Q. occurs, averaging 378 c.c. per kilo. The figure is somewhat greater than for the CO_2 lost from eviscerated cats. Blood and muscle consistently lose CO_2 in over-ventilation, but the amount so accounted for is only 10 p.c. of the total loss calculated from respiratory measurements.

Exp. 1. Over-ventilated cat. 29. i. 29.	
Weight	1920 g.
Time over-ventilated	2 hr. 50 min.
Respiration.	
Before over-ventilation	0·555 litres per min.
During over-ventilation	1·10 „ „
R.Q. before	0·72
R.Q. after	0·81, 0·90, 1·0, 1·0, 1·39, 1·36, 1·19
CO_2 expired	2554 c.c.
CO_2 calculated from O_2 consumption at R.Q. = 0·72	1610
Extra CO_2 expired	940
Blood.	
Initial CO_2 content	39 vol. p.c.
Final CO_2 content	22 „
CO_2 loss = 0·17 × 0·05 × 1920 =	17 c.c.
Muscle.	
Initial CO_2 content	25 vol. p.c.
Final CO_2 content	16 „
CO_2 loss = 0·09 × 0·44 × 1920 =	76 c.c.
Total loss from blood and muscle =	93 c.c.

TABLE I. Summary of Experiments on over-ventilated cats.

Date	Weight g.	Time of over-ventilation period h. m.	Respiration during over-ventilation: Initial R.Q.	CO_2 expired c.c.	O_2 consumed c.c.	Average R.Q.	Excess CO_2 c.c. per kilo	CO_2 lost: From muscle c.c. per kilo body weight	CO_2 lost: From blood c.c. per kilo body weight	P.c. of CO_2 loss accounted for
17. xii. 28	1550	4 51	0·72	4749	5542	0·86	480	35	6·6	8·6
18. xii. 28	2350	4 15	0·82	3345	3024	1·10	370	17	8·0	7·0
29. i. 29	1920	2 50	0·72	2554	2240	1·14	490	40	8·8	9·9
30. i. 29	1250	3 0	0·74	2279	2777	0·82	175	31	7·5	21·8

The CO_2 loss from blood cannot possibly be very great. The total CO_2 in the blood of a cat, assuming a concentration of 40 volumes p.c. is only 20 c.c. per kilo, and the actual loss from blood is about 16 volumes p.c. in each experiment, or 8 c.c. per kilo, which is only 2 p.c. of the total loss calculated. The muscles listed in Table II lost an average of about 7 volumes p.c. of CO_2, or 31 c.c. per kilo, *i.e.* 8 p.c. of the total CO_2 loss. Here again it is plain that the loss of all muscle CO_2, 88 c.c. per kilo, would not go far toward explaining the calculated extra CO_2 expired. To calculate the total muscle CO_2, the muscles of a cat have been taken as 44 p.c. of the whole weight (1).

TABLE II. Muscle and blood changes during over-ventilation.

Experiment	Total CO_2 in muscle (in vols. p.c.): Before	After	Total CO_2 in arterial blood (in vols. p.c.): Before	After	Change in muscle / Change in blood
17. xii. 28	18·8	11·0	36·9	24·0	0·58
	19·9	12·6			
18. xii. 28	16·9	13·0	35·7	19·8	0·28
	17·4	12·2			
5. ii. 29	21·7	14·1	41·9	26·4	0·49
29. ii. 29	27·3	18·6	39·7	23·0	0·56
	27·1	17·1			
30. i. 29	26·1	16·6	35·7	19·8	0·48
	24·3	18·5			
				Average	0·48

In three experiments the final CO_2 tension of arterial blood had fallen from an initial 40, 49 and 35 mm. Hg to 13, 15 and 12 mm. Hg respectively. From four experiments in which there were three or more determinations of total CO_2, it appeared that the blood had reached its minimum CO_2 content during over-ventilation within a half-hour. The R.Q.'s usually continued to rise for from 2 to 4 hours. There was no sign of approaching equilibrium or a minimum CO_2 content at a new low CO_2

tension in other tissues than blood, although it might have been reached if the over-ventilation had been less severe.

There remains a balance of about 90 p.c. of the calculated extra CO_2 to be accounted for, about 340 c.c. per kilo body weight. In three over-ventilated cats the tibias contained about 10 c.c. of CO_2 per gram, but the difference between normal and over-ventilated bones did not exceed 3 p.c. The bones make up about 25 p.c. of the cat's weight (1) and contain, on the basis of the single bones analysed, about 2500 c.c. of CO_2 per kilo. If the extra CO_2 had been derived from bones, they would have lost 13 p.c. of their CO_2. The loss of even half that amount should have been detectable.

Ventilation with CO_2 rich mixtures.

The converse of over-ventilation experiments are the following ones in which cats under amytal were caused to breathe mixtures containing from 6 to 9 p.c. CO_2. CO_2 mixtures were prepared in a spirometer, usually with oxygen added to approximate the oxygen tension of atmospheric air. After collecting normal blood, air, muscle and bone samples, the animals were curarized and the spirometer mixture led through a metre and the respiration pump. As before, samples of expired air were taken at intervals throughout the experiment. Samples of arterial blood, muscle and bone were secured at the termination of the experiment. From the oxygen consumption calculated from the results of the analysis of each sample, the CO_2 of metabolism was calculated as if the original R.Q. prevailed. The p.c. CO_2 expired in the sample was considered to obtain for the preceding period. Subtracting the expired CO_2 from the CO_2 of metabolism plus the CO_2 inspired showed the amount of CO_2 retained. Actually, as the CO_2 content of expired air rose in successive samples, the amount expired in the early periods is less than the amount indicated by the sample terminating the period, making the calculated retained CO_2 too low.

A typical experiment is outlined as Experiment 2. At the start of ventilation with 7·02 p.c. CO_2, the CO_2 of the expired air was less than that of inspired air. Gradual accumulation of CO_2 in the tissues produced a CO_2 tension sufficient to cause the normal CO_2 elimination, and the R.Q. returned to its original value. No observed characteristic of metabolism was different at the end of the experiment from the start, and the tissue CO_2 tension was evidently in equilibrium with the new higher CO_2 tension. Blood and muscle CO_2 content were consequently increased.

Exp. 2. Cat breathing 7·02 p.c. CO_2. 21. i. 29.

Weight	2850 g.
Respiration.	0·585 litre per min. (22°)
R.Q. before breathing CO_2 mixture	0·75
R.Q. after breathing CO_2 mixture	0·69[1], 0·29, 0·39, 0·37, 0·47, 0·64, 0·76, 0·75
Average	0·51
CO_2 expired (excess over inspired)	3570 c.c.
CO_2 calculated from O_2 consumption at R.Q. = 0·75	5250 c.c.
CO_2 retained	1680 c.c.
Blood.	
Initial CO_2 content	33·7 vol. p.c.
Final CO_2 content	49·4 „
CO_2 retained = 0·16 × 0·05 × 2850 =	23 c.c.
Muscle.	
Initial CO_2 content	19·5 vol. p.c.
Final CO_2 content	34·1 „
CO_2 retained = 0·15 × 0·44 × 2850 =	188 c.c.
Retention by blood and muscle	211 c.c.

Four such experiments are summarized in Table III. Two of the experiments, lasting for over 4 hours (January 21 and March 19) show an exact return to the original R.Q. In one experiment (March 18) on a decapitate cat the R.Q. returned to 0·78 in 2 hours 35 minutes, compared with a normal of 0·90. The normal rather high R.Q. is the average of two samples of which the second was the lower at 0·86, so that the true preliminary R.Q. was perhaps less than 0·90. The experiments on January 21 and March 19 indicate certainly that final equilibrium has been attained at the higher CO_2 tension. In the experiment of March 18 we may say that equilibrium is practically attained. The first experiment (December 3) lasted for only 2 hours, during which time the R.Q. did not return to the starting value. Evidently equilibrium was not reached.

TABLE III. Summary of experiments on cats ventilated with CO_2 rich air.

Experiment	Weight g.	Duration of tank air breathing h.	m.	Initial R.Q.	Respiratory measurements: CO_2 expired c.c.	O_2 consumed c.c.	CO_2 retained c.c. per kilo	Average R.Q.	Tissue CO_2 gain: In muscle c.c. per kilo body weight	In blood c.c. per kilo body weight	CO_2 retention accounted for p.c.
23. xii. 28	1550	2	0	0·64	660	3470	1000	0·19	26·4	2·96	2·9
21. i. 29	2850	4	40	0·75	3570	6920	568	0·50	66·0	8·0	12·5
18. iii. 29	3180	2	35	0·90	1425	2156	162	0·66	59·0	7·8	42
19. iii. 29	4720	4	0	0·83	5900	8540	250	0·69	74·5	4·0	31

[1] CO_2 of expired air was less than inspired by an amount giving this ratio to the oxygen consumed.

In the last three experiments, in which equilibrium was attained, the average blood CO_2 content increase was only 6 c.c. per kilo of cat; not enough to be significant. The CO_2 retained by muscle was quite large, averaging 67 c.c. per kilo, and with that absorbed by the blood accounting for 13 to 42 p.c. of the amount retained.

As from 58 to 87 p.c. of the retained CO_2 cannot be located, it is well to consider the rate of CO_2 absorption by blood and muscle. In two experiments where several blood samples were taken, the maximum CO_2 content was already present in the first samples analysed, after ½ hour and 1 hour respectively. Consequently the arterial blood maximum was reached rapidly. It seems unnecessary to figure on the attainment of the maximum CO_2 content in venous blood, for the quantity of CO_2 involved by the blood changes was always relatively small. Venous blood equilibrium would only be reached when the R.Q. returned to its starting value.

We have not been able to follow the rate of increase in muscle CO_2 content in many experiments. However, by taking successive muscle samples in two experiments it was evident that the muscle CO_2 content reached its maximum after blood and before the process of CO_2 absorption was complete. Establishment of muscle saturation preceding return to respiratory equilibrium would indicate an absorbing system for the unexplained CO_2 distinct from blood and muscle.

The critical factor in determining CO_2 absorption by tissues is the CO_2 tension. In three experiments the arterial blood CO_2 tension increased

TABLE IV. Muscle and blood changes during ventilation with CO_2 rich air.

Experiment	Total CO_2 in muscle (in vols. p.c.)		Total CO_2 in arterial blood (in vols. p.c.)		Change in muscle / Change in blood
	Before	After	Before	After	
3. xii. 28	27·2	33·7	36·1	42·4	1·13
	26·1	33·9			
4. xii. 28	15·4	26·6	26·1	42·1	0·68
	15·8	26·3			
9. xii. 28	27·4	36·6	44·8	53·0	1·02
	28·5	36·0			
21. i. 29	20·9	36·9	33·7	49·4	1·0
	21·1	36·5			
19. ii. 29	28·8	48·5	47·3	55·2	1·96
	30·9	42·3			
18. iii. 29	18·8	33·5	30·2	47·3	0·88
	19·5	35·1			
19. iii. 29	19·6	36·6	34·3	42·2	2·28
	18·5	37·6			
				Average	1·28

from 20, 25 and 30 mm. to 30, 67 and 51 mm. respectively. If we knew the true CO_2 tension in muscle we could make a CO_2 dissociation curve, but arterial blood represents only the lowest possible muscle CO_2 tension. Comparison of the amounts of CO_2 absorbed per unit of blood and muscle (Table IV) shows that muscle gains on the average 1·2 times as much CO_2. Consequently muscle CO_2 capacity exceeds that of blood toward increasing CO_2 tension, while for over-ventilation the relation was reversed, and muscle CO_2 lost only half as much CO_2 per c.c. as blood.

The changes in CO_2 content of these two tissues account for only one-third of the absorbed CO_2. As in the eviscerated and over-ventilated cats, where bone was regarded as the likely source of CO_2, in the high CO_2 cats it was considered as a possible site of retention. Results of analysis of a number of bones are given in Table V. The analysis of corresponding bones before and after CO_2 breathing showed more frequently than not a gain in CO_2 content. The average amount of CO_2 gained, allowing 25 p.c. for bone weight, was about 60 c.c. per kilo. After allowing for blood and muscle in the three experiments in which equilibrium was reached, an average of 260 c.c. of CO_2 per kilo remained to be explained. The retention of 60 c.c. of CO_2 by the bones is enough to suggest that bones constitute an important site of retention of the unexplained absorbed CO_2.

TABLE V. Bone CO_2 content after ventilation with CO_2 rich air.

Experiment	Bone	c.c. CO_2 per g.			Ca mg. per g.		c.c. CO_2 per g. Ca		Loss (−) or gain of CO_2 per g. Ca
		Before	After	Δ	Before	After	Before	After	
13. ii. 29	Tibia proximal	9·91	10·18	0·27	135	141	73·4	72·2	−1·2
	10th and 11th ribs	12·02	11·36	−0·68	162	155	74·3	73·4	−0·9
19. ii. 29	Fibula	15·31	16·72	1·41	—	—	—	—	—
18. iii. 29	Distal Tibia	12·22	12·51	0·29	184	196	66·5	64·0	−2·5
	Fibula	14·50	14·33	−0·17	224	224	64·7	64·1	−0·6
19. iii. 29	Distal Tibia	13·20	12·99	−0·21	196	192	67·5	67·7	0·2
25. iii. 29	Distal Tibia	12·06	12·46	0·40	186	188	64·9	66·3	1·4
27. iii. 29	Distal Tibia	13·24	13·34	0·10	193	204	68·7	65·5	−3·2
	Fibula	14·62	15·21	0·49	220	228	66·5	66·8	0·3

These analytical differences are not, however, convincing. It has been shown that the ratio $\frac{Ca}{CO_2}$ in bones of rats is a characteristic of their age (4). In an alteration of the CO_2 content the calcium content is a natural reference point, and changes in CO_2 content might appear more clearly when expressed in terms of CO_2 per g. Ca. Referring bone CO_2 to calcium does not show a gain in CO_2 but, on the contrary, more frequently implies a

loss. As in the eviscerate cat, it appears that the bones may serve as reservoirs of labile CO_2, but the evidence is not conclusive.

Possible alternative sites of retention or loss of CO_2 may be considered together, as they probably involve similar processes. Entrance of significant amounts of extraneous CO_2 into these over-ventilated cats breathing outside air is not possible. But CO_2 might escape from the cats breathing CO_2 rich air. In two experiments cats were sealed in a metal box and ventilated with about 7 p.c. CO_2 through a tracheal cannula sealed in the box. If CO_2 escaped through the skin or small exposed area of the trachea, it would be caught in the box. CO_2 free air was then drawn through the box and passed through sulphuric acid and weighed absorption tubes. During 6 hours 33 mg. of CO_2 were collected, *i.e.* a loss from the skin of about 17 c.c. of CO_2. In the second experiment one leg was amputated, in order to see if CO_2 escaped more readily when the conditions were similar to those of our experiments. 8 mg. or 4 c.c. were collected in 2 hours, while the blood CO_2 content increased from 36·7 to 44·7 volumes p.c. Apparently the small quantity of CO_2 that can be lost through the skin has no significance in contrast to the large amount of CO_2 retained. These results agree with the findings of Shaw (7).

Examination of several viscera in two normal animals showed that their CO_2 content was only slightly higher than that of muscle, as is indicated in Table VI. As all of the viscera weigh less than half as much as all of the muscles, a CO_2 retention in the viscera corresponding to that in muscle would amount to about 30 c.c. per kilo. It is probable that changes in the viscera would account for nearly 10 p.c. of the retained CO_2, but the unexplained balance would not be significantly reduced. The loss from the viscera during over-ventilation, if comparable to the muscle loss, would be somewhat less.

TABLE VI. CO_2 content of soft tissues of normal cats. Average for two cats.

Tissue	CO_2 in c.c. per 100 g.
Liver	32
Brain	27
Kidney	24
Ileum	31

Urea formation might be considered a cause of CO_2 retention. Shaw (5) considered it ruled out by finding CO_2 retention unchanged after tying off the ureters, but that proceeding would not prevent the formation of urea. We have made no attempt to rule out possible excretion because the rôle of CO_2 in the process of urea formation is uncertain[1].

[1] This possibility has now been investigated.

Conclusions.

It is apparent that an increase or a decrease in alveolar CO_2 tension causes respectively a decrease or an increase in CO_2 elimination. The altered rate of CO_2 elimination can cause a great change in R.Q. for several hours at least. There is no reason for suspecting a change in the kind of material oxidized, although the possibility is not ruled out. It is well known that changes in blood CO_2 content follow closely after alteration in the alveolar CO_2 tension, but the total amount of blood CO_2 is not sufficient to cause much change in R.Q. The muscle CO_2 is labile, like that of blood, and contributes roughly four times as much as blood to the change in R.Q. following altered alveolar CO_2 tension. But the combined changes in blood and muscle do not account for half of the change in R.Q.

When it is considered that CO_2 may be either retained or given off according to the alveolar CO_2 tension, it is likely that the tissue carbonates and bicarbonates constitute the buffer or reserve system. After proper allowance is made for the blood, muscles and viscera, bones are the only tissue with a sufficient CO_2 content to serve as the remaining part of the reserve system. Loss or gain in bone CO_2 was not established during these acute experiments, but the large amount of CO_2 present in bone makes it difficult to say to what extent the small changes required in bone carbonates would be detectable. It is still possible that other physiological adjustments of the tissues, such as urea formation, are partly responsible for the change in R.Q.

The extent of the blood and muscle CO_2 reserves has been traced, the visceral reserves indicated, and the bones shown adequate for the remainder. There is no evidence for a change in oxidative metabolism, and good evidence that a tissue reserve or buffering system causes changes in the R.Q. following every alteration in the alveolar CO_2 tension.

The picture of changes in R.Q. and in the part contributed to those changes by blood and muscle is similar in eviscerated and over-ventilated cats. Consequently it does not seem proper to conclude that the R.Q. of the eviscerated preparation is a true indicator of metabolism until the tissue CO_2 reserves are examined and either appropriate corrections can be made or the experimental procedure prevents loss of tissue CO_2.

SUMMARY.

During over-ventilation of cats, 375 c.c. of CO_2 per kilo may be expired in excess of the amount which would be necessary to maintain the original type of metabolism. Of this extra CO_2, about 8 c.c. per kilo comes from blood, about 31 c.c. from muscle.

During ventilation with CO_2 rich mixtures about 375 c.c. of CO_2 per kilo may be retained. Blood retains about 6 c.c. per kilo, muscle about 67 c.c.

It is possible that bone may account for much of the unexplained retained CO_2, but the results of our analyses give no convincing support to this view.

The large changes in R.Q. following artificially altered alveolar CO_2 tension have not been entirely assigned to definite tissues by analytical methods. Judging by the characteristics of the tissue changes observed, the R.Q. changes originate in alteration of the tissue CO_2 reserves.

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THE RESPIRATORY MOVEMENTS IN GRADUAL HYDROCYANIC ACID POISONING.

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LUMSDEN(3) has described various types of respiratory movements obtained by cutting through the brain stem at different levels. The present experiments have been carried out to ascertain whether the types of respiratory movements described by Lumsden are found during the different stages of HCN poisoning.

The centres which Lumsden assumes in the mammal are (1) the pneumotaxic centre, (2) the "apneustic" centre, (3) the expiratory centre, and (4) the gasping centre in this order, from above downwards. He considers that normal respiration is under the control of the top two centres, the other two centres only functioning in abnormal conditions. Inspiration is under the control of the "apneustic" centre and the nervous control of normal respiratory movements consists of alternate release and inhibition of the apneustic centre. This alternate release and inhibition of the apneustic centre is effected by the pneumotaxic centre which is the centre most susceptible to changes in CO_2 and acid in the blood. The lower two centres are normally inhibited by the upper two centres.

By cutting between the top two centres Lumsden was able to get respiration of the "apneustic type," *i.e.* an inspiratory tonus which persisted until deoxygenation of the blood brought the tonus to an end. By cutting the brain stem lower down gasps only were obtained. The effects of the possible expiratory centre are not obtained so easily.

Lumsden also found that the same effects were obtained in bulbar anæmia obtained by shutting off the blood supply to the brain. As the effect of HCN is to prevent oxidation the action of HCN ought to be similar to the effect of anæmia, provided that death does not take place too rapidly. If the brain stem dies gradually under the influence of HCN the various types of respiratory movements can be compared with those claimed by Lumsden.

Evans(2) has studied the effect of injections of sodium cyanide on

the respiration and finds a marked increase in the respiratory rate and amplitude at the beginning of cyanide poisoning with a subsequent reduction in the rate of respiratory rhythm. As Evans's record of the respiration was made by means of a tambour on the tracheal tube, only movements of the air in the trachea are recorded and inspiratory tonuses would not be recorded (Lumsden(6,7)). Evans also remarks that different parts of the central nervous system are affected to a different extent and that small doses of cyanide seem only to affect the cerebrum.

Bunge(1) described the effect on the respiration of breathing cyanogen. His experiments, like those of Evans, are inadequate to show whether or not "apneuses" were obtained.

Experimental procedure.

The respiratory movements have in most cases been registered by the method advocated by Lumsden, *i.e.* glycerine tambours on the thorax and the abdomen. In some experiments a tracheal tube has also been used, and with a few experiments on rabbits, Head's method of recording the movements of a slip of diaphragm muscle has been utilized. The majority of the experiments have been carried out on cats.

The tambour for use in the thorax was of the largest possible diameter (8·5 cm.) to give a maximum movement to the recording tambour. The abdominal tambour was much smaller (5·5 cm. diameter) as the record here is easier to obtain. Each tambour was made of a double layer of rubber with glycerine between the rubber surfaces, so that the surface to be placed on the thorax or abdomen was always convex. The convexity was not very marked, and the centre of each tambour was about 10 mm. above the level of the sides. The thoracic tambour was placed on the right side of the thorax to reduce the interference of the heart beat, and the abdominal tambour was placed on the mid-line of the body, where the respiratory movements are maximal. The tambours were fixed in position by binding them with bandages, the pressure not being great enough to interfere with the respiratory movements. The whole of the abdomen was enclosed in bandages so that it was under equal pressure, otherwise the position of maximum movement might have been altered. The records were obtained by attaching these tambours by semi-pressure tubing to Brodie tambours which recorded on the kymograph. On both the abdominal and thoracic recording tambour inspiration was indicated by an upstroke and expiration by a down stroke.

The HCN was used in gaseous form and was set up in a respiration chamber of 6·3 cu. metres capacity. In most of the experiments the

animal was inside the chamber and breathed through the nose and occasionally through a tracheal tube. In experiments where air was pumped into the lungs and a tambour placed on the tracheal tube, the animal was outside the chamber and breathed the air in the chamber on an external circuit *via* valves and a tracheal tube. This was also the case where Head's method was used on rabbits. The kymograph was outside the chamber, connection being made between the glycerine tambours on the animal and the recording tambours by tubes which passed through orifices in the glass, the chamber however being still air-tight.

The gaseous HCN was produced by pouring liquid anhydrous HCN in a trough and blowing air over it by means of a fan. In each experiment the concentration was analysed by drawing off 25 litres and absorbing the HCN in caustic soda, the solution being then titrated against silver nitrate. The error due to the breathing of the animal was very small, although in a few experiments there might have been a reduction in the HCN concentration owing to an observer having been in the chamber for a considerable time.

The cats were all anæsthetized by either chloroform or chloroform and ether, whilst in the case of rabbits sodium luminal was injected and the rabbit then chloroformed. The animal was placed in the chamber without any HCN and a record of the normal respiration taken. The vagi were then cut and the effects on the respiration were observed. The HCN atmosphere was then set up and the record of the respiratory movements obtained until the animal died. The vagi were cut to aid the formation of apneuses, as Lumsden found them difficult to obtain in bulbar anæmia with the vagi intact. A number of experiments were done with the vagi intact.

Effect of HCN on the cat's respiration.

Twenty-five experiments were done on cats, the concentration of HCN varying from 0·38 g. per cu. metre to 0·23 g. per cu. metre. The most usual concentration was of the order of 0·30 g. per cu. metre. The cats varied very much in the length of time they survived, but with the lower concentrations the cat could be got to live for 2 hours and generally lived for 30 minutes.

The typical effect of HCN is shown on the kymograph tracing. In the figures given the thoracic tracing is the upper one and the abdominal tracing the lower one. As the animal was breathing HCN the effects developed gradually as the HCN accumulated in the blood stream. By this means the effects were spaced out, and by varying the concentration of HCN the time between the onset of HCN poisoning and death could

be extended so that each phase was clearly presented. The disadvantage of injecting HCN solution was that the maximum effect was obtained very rapidly and only the later phases could be examined with any certainty.

In the tracing with a chloroformed cat the first effect of the HCN was to cause a slight increase in the amplitude of the respirations, but not in the rate, although the rate might increase to some extent immediately afterwards. Fig. 1 shows the first effects of the HCN on a cat with the vagi cut.

Fig. 1. Beginning of effect of HCN (Cat). Top: thorax. Bottom: abdomen. Time in secs. HCN = 0·37 g. per cu. metre.

Next the respiration tended to become irregular, short periods of inspiratory tonus being mixed with normal respirations. During this phase also the record was quite liable to be unreliable owing to the HCN causing muscular movements in the cat. These movements did not occur when the cat was heavily under the anæsthetic. Inspiratory tonuses now became more marked and for a time respiration is all of the "apneustic" type and increasing in amplitude. These are shown in Fig. 2. The "apneuses" showed on both the thoracic and abdominal tracings and were very rarely sustained contractions, there being some slight movements of the lever in the inspiratory position.

The lengths of the "apneuses" differed widely, the longest obtained being 20 seconds, although normally they were of 6 to 8 seconds' duration. The slight movements at the peak of an "apneusis" always corresponded to inspiration when the experiment was tried with a tambour on the tracheal side tube.

The length of time of the "apneusis" gradually diminished as the

Fig. 2.

Fig. 3.

Fig. 2. "Apneuses" (Cat). Top: thorax. Bottom: abdomen. Time in secs. HCN = 0·37 g. per cu. metre.

Fig. 3. "Apneustic gasps (Cat). Top: thorax. Bottom: abdomen. Time in secs. HCN = 0·37 g. per cu. metre.

HCN became more effective and a phase of "apneustic gasps" was obtained, *i.e.* gasps with two or three peaks as shown in Fig. 3. The change from "apneuses" to gasps was gradual, the last signs of "apneuses" being gasps with two peaks. Gasping then followed, the amplitude of the gasps being generally much greater than that of respirations, but after a time the amplitude diminished fairly quickly and the animal died. Gasps are shown in Fig. 4 just before death. Figs. 1, 2, 3, and 4 are all

from the same cat: Fig. 2, 6 minutes from the setting up of the HCN; Fig. 3, 17 minutes and Fig. 4, 30 minutes.

If the animal died too rapidly the "apneuses" might not be marked and only appeared as a few gasps with double peaks.

When gasping took place the movements were at their maximum amplitude, the gasp appearing to be a sudden maximal contraction of the inspiratory muscles. The mouth always opened during gasping irrespective of whether the animal was breathing *via* the nose or a tracheal

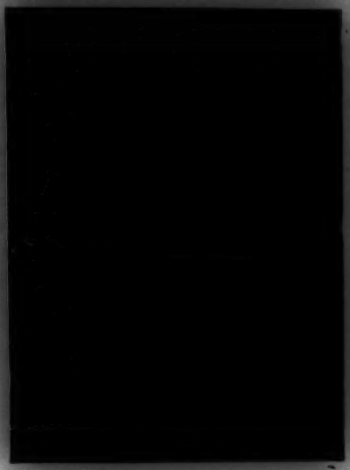

Fig. 4.

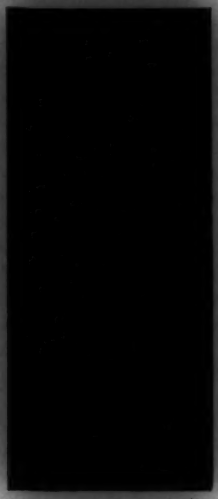

Fig. 5.

Fig. 4. End of gasping (Cat). Top: thorax. Bottom: abdomen. Time in secs. HCN = 0·37 g. per cu. metre.

Fig. 5. Gasping: rise in thoracic tracing (Cat). Top: thorax. Bottom: abdomen. Time in secs. HCN = 0·32 g. per cu. metre.

tube. The mouth was also open during some of the longer apneuses. When a gasp took place the abdominal lever rose before the thoracic lever and reached the summit first. In many cases the beginning of a rise of the abdominal lever coincided with a slight downward movement below the base line of the thoracic lever, after which the thoracic lever rose rapidly. This is the case in Fig. 4. The fact that the abdominal lever moved first

and was sometimes accompanied by a fall of the thoracic lever might be due to the diaphragm having contracted slightly before the intercostal muscles.

Besides the effects described in many cases temporary rises of either the thoracic or abdominal lever have occurred (Fig. 5). The lever rose to a maximum and then fell back to normal gradually, the total time having been of the order of 1 or 1½ minutes, with the respirations or gasps superimposed.

The normal effects of HCN on the cat's respiration reproduce types of respiratory movements similar to those obtained by Lumsden by section of the brain stem. The effects are obtained in the same order as by cutting through the brain stem in sections from above downwards. Lumsden's experiments were done mostly on cats, so the experiments with HCN can be compared with them.

The effects show a gradual changing of the respiration from a normal to an "apneustic" type. This "apneustic" phase is not as marked as when obtained by sections in the brain stem, as probably the whole of the brain is affected to some degree by the HCN. The gasping phase at the end is almost identical with the phase obtained by cutting low down in the brain stem. The "apneustic" phase and the gasping phase seem to be running together for a short time as shown by "apneustic" gasps obtained. No trace of an expiratory centre was found.

Irregularities are sometimes found in the abdominal tracing during the early stages of the gasping phase. These will be discussed later.

Effect of HCN on the rabbit's respiration.

Lumsden(3) has pointed out that rabbits differ from cats in the effect obtained by cutting through the brain stem. The effect of the vagus is more marked and the positions of the sections show differences. Their reaction to HCN is also different in some respects.

Twelve experiments were done on rabbits, the HCN concentrations varying from 0·31 g. per cu. metre to 0·42 g. per cu. metre, with an average of 0·38 g. per cu. metre (*i.e.* a higher concentration than for cats). The rabbits could easily be made to survive 30 minutes, and would appear to survive indefinitely in the case of lower concentrations.

The experiments were carried out in the same way as the experiments on cats, only 0·2 g. of sodium luminal was injected intravenously half an hour before the chloroform was administered. The same glycerine tambours were used in both cases.

The chief difference between the effect on rabbits and that on cats

was that "apneuses" were not obtained except in rare cases. On only one experiment with a rabbit were "apneuses" obtained.

In the rabbit the preliminary effects of the HCN, *i.e.* a slight increase in the amplitude of the respirations, were the same. The respiration, after some irregularities, settled down to a steady rhythm, but after a time the amplitude decreased and there was a cessation of respiration. This pause extended sometimes for 3 or 4 minutes and the animal appeared to be dead. Gasps, however, of small amplitude then began to appear. In some cases at the beginning of gasping the abdominal lever gave an upward stroke, whilst the thoracic lever registered a down stroke suggesting contractions of the diaphragm alone of sufficient power to suck in the thorax. The gasps increased in amplitude up to a maximum and then began to decline, whilst at the end the thoracic tracing sometimes registered a small down stroke only. Occasionally there would be a short pause between two sets of gasps. Fig. 6 shows the beginning, end and

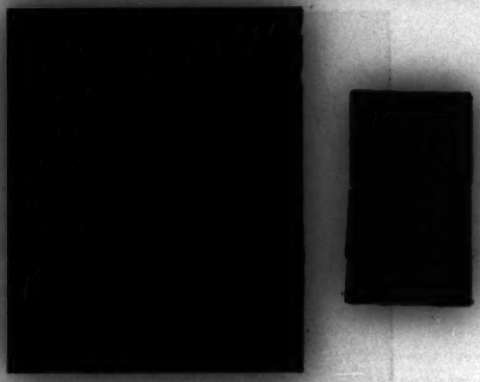

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Fig. 6. Gasping (Rabbit). Top: thorax. Bottom: abdomen. Vagi cut: (1) Beginning. (2) Late middle period. HCN = 0·42 g. per cu. metre.

the middle period of gasping in a rabbit. It will be noticed that at the beginning the abdominal and thoracic levers register in opposite directions.

A pause between the breathing and the gasping was not always obtained, but seemed to be the general rule.

Effect of pumping air when animal is affected by HCN.

When air was pumped in and out of a cat's lung during the gasping stage under HCN poisoning, the pumping did not produce apnœa although the cat was not breathing quickly. The muscular signs of gasping such as opening the mouth, etc., continued during the action of pumping, although the rate of ventilation was considerably quicker than the normal gasping. The effect was the same in a rabbit where it was shown by using Head's method of recording the contractions of a slip of diaphragm muscle. Lumsden(5) states that in an animal gasping as a result of a section of the brain stem, CO_2 and O_2 will affect the rhythm and amplitude of the gasps, but apnœa cannot be produced. Thus the gasping produced by HCN resembles the gasping produced by section of the brain stem in that apnœa cannot be produced by over-ventilation.

Over-ventilation did produce a change in the character of the gasps

Fig. 7. Double abdominal movements (after pumping air) (Cat). Top: thorax. Bottom: abdomen. Vagi cut. Time in secs. HCN = 0·28 g. per cu. metre.

in the cat, but not the rabbit. The abdominal lever now registered two strokes to one on the thoracic lever, the extra stroke taking place whilst the thoracic lever was at rest. The movements were quite regular and gradually passed off, but could be reproduced easily. The extra movement was sometimes of larger amplitude than the gasp. These are shown in Fig. 7. They sometimes appeared in the normal tracing of HCN poisoning between the "apneustic" and the gasping state, and occasionally preceding an "apneusis." The movement seemed to be due to a contraction of the abdominal muscles, but it is not accompanied by any passage of air in or out of the trachea.

In an animal, cat or rabbit, which had been allowed to reach the gasping stage, artificial ventilation with the same HCN mixture produced rapid failure of all respiratory movements. Presumably more HCN was absorbed, and the rate of detoxification in the body could not keep pace with the absorption.

Influence of the vagus on "Apneuses."

Lumsden(7) has pointed out that the "apneuses" were difficult to obtain in bulbar anæmia if the vagi were intact. Apneuses were not very marked in cats with intact vagi during HCN poisoning. Short "apneuses," however, do appear, and occasionally "apneuses" appear which pass off again leaving long respirations. If the vagi were cut after the onset of HCN poisoning "apneuses" followed, though not always immediately, depending on the degree to which the cat was affected. The vagi appeared to inhibit "apneuses" as found by Lumsden in sections of the brain stem.

Conclusions and summary.

The effect of HCN on the respiration gives the same series of phenomena as found by Lumsden by cutting sections at various levels of the brain stem. The effects are obtained in the same order as those given by the cuts from above downwards.

The action of HCN on cats is what would be expected by assuming the successive elimination of the "pneumotaxic" and "apneustic" parts of the respiratory mechanism. In the rabbit both these mechanisms, if they exist separately as such, seem to be eliminated together, but gasping is very similar to gasping in cats.

Over-ventilation with air does not produce apnœa in a gasping animal, nor do the respiratory movements stop during the act of over-ventilation. In a cat the form of the gasps is altered after artificial ventilation.

Artificial ventilation with the HCN mixture produces failure of the respiratory movements.

Cutting the vagi appears to aid the formation of apneuses produced by HCN.

I must thank Prof. J. Barcroft, at whose suggestion the work has been carried out, for much valuable advice and criticism.

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STUDIES ON WATER DIURESIS.

Part III. A comparison of the excretion of urine by innervated and denervated kidneys perfused with the heart-lung preparation.

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In the previous paper of this series (3), as a result of experiments upon the excretion of urine from innervated and denervated kidneys in hypophysectomized and decerebrated dogs, one of us suggested that under normal conditions the pituitary body is not concerned with the regulation of the renal excretion of chlorides and water; and that the "ingravescent polyuria" characteristic of the isolated kidney perfused by the heart-lung preparation is not entirely due to the disappearance of pituitrin or pituitrin-like substances from the circulating blood, but rather the result of complete denervation consequent upon the removal of the kidney for perfusion. It therefore seemed advisable to re-investigate the excretion of urine from the isolated kidney, in order to determine to what extent denervation could be held responsible for the increased urine flow and reduced chloride output.

Experimental technique.

The only fundamental difference in the final procedure adopted in these experiments and that employed in the preparation of a heart-lung circuit as described by Starling and Verney (4) was that, instead of completely excising the kidney, it was perfused *in situ* by means of a cannula placed in the aorta distal to the renal artery; mixing of the perfused blood with that of the animal being prevented by appropriately placed ligatures. As the blood left the kidney, through a slit in the renal vein, it was collected from the abdominal cavity by a tube piercing the dorsal wall of the animal, and returned to the venous reservoir of the heart-lung preparation by the automatic venous return pump described by us elsewhere (1). Adequate precautions were taken to avoid the blood

of the heart-lung-kidney circuit coming into contact with that of the kidney dog, and the renal nerves were left undisturbed as far as was possible. We wish to emphasize the following points of our technique:

(1) The establishment of the heart-lung circuit from 30 to 60 minutes before the kidney was switched into the circuit.

(2) The fact that no saline or urea were added to the blood circulating through the kidney.

(3) At no period was the renal blood flow cut off or the renal blood-pressure lowered during the transfer of the kidney from its own circulation to that of the heart-lung preparation.

(*a*) *The heart-lung circuit.*

Details of the heart-lung circuit were altered to ensure greater stability in the temperature of the blood, reduction in the volume of blood in circulation, and greater facility in recording blood flows and blood-pressure. The heating coil in the main circuit was eliminated and the remainder of the apparatus, including the venous reservoir, enclosed in a continuously stirred water bath, the temperature of which was accurately controlled by an electric thermo-regulating system. The venous blood leaving the kidney was passed through a heating coil in this bath before being returned to the main venous reservoir. These slight modifications ensured a regular and easily adjustable blood temperature. The usual precaution of respiring the animal with warmed air to avoid heat loss from the blood circulating through the lungs was also observed. It was usual to so time the completion of the heart-lung circuit that 30 to 60 minutes were available before shunting the kidney into the circuit. This allowed time for the removal of any "vasotonins" in the freshly defibrinated blood, and also for the disappearance of any posterior pituitary substance which may have been normally present.

(*b*) *The kidney circuit.*

After chloralose anæsthesia a mid-line incision was made in the abdomen of the kidney dog and all of the intestines, from the rectum to the junction of the tail of the pancreas with the duodenum, carefully removed between double ligatures. The spleen was also excised and the right kidney ligatured off but left *in situ*. The fascia binding the left kidney to the dorsal wall of the peritoneal cavity was then slit at its lateral attachment and the kidney itself gently retracted towards the right side. This allowed free access to the aorta and exposed the renal nerves. The aorta was then cleared and lifted and all branches within 2 cm. above and

below the origin of the left renal artery tightly ligatured. Three ligatures were then loosely placed around the aorta, one proximal and two distal to the renal artery, care being taken to avoid including any of the small nerves which frequently pass along the sheath of the aorta to the kidney, *via* the renal artery. Another loose ligature was placed about the renal vein about 1·5 cm. from its origin from the renal pelvis. Finally a ureteral cannula was inserted and the drainage tube of the venous return system thrust through the dorsal wall of the abdominal cavity lateral to the kidney. The kidney was then returned to its normal position, and the carotid blood-pressure and urine flow recorded before switching over to the heart-lung circuit.

The transference of the kidney to the heart-lung circuit was effected without reduction in the arterial pressure or stoppage of the renal blood flow in the following manner. A clip was placed over the aorta distal to the renal artery and a cannula, connected with the arterial side of the heart-lung circuit, inserted. The pressure at the head of this cannula was then adjusted until it was approximately that recorded from the carotid artery of the kidney dog. After placing a small amount of heparin in the abdominal cavity the renal vein was quickly ligatured and slit in such a manner to allow free exit of blood from the kidney. Upon removal of the aortic clip blood was capable of entering the kidney either from the heart-lung circuit or from the kidney dog itself. The transfer was then completed by tying the aortic ligature "cephalad" to the renal artery.

Complete isolation of the kidney circuit can only be obtained when all hæmorrhage is carefully controlled and all branches leaving the aorta in the region of the renal artery tightly ligatured. It was usual to increase the kidney perfusion pressure above that in the animal in order to ensure that no blood should find its way through anastomotic channels into the heart-lung-kidney circuit.

Results.

(*a*) *A comparison of the renal blood flow and urine excretion before and after denervation.*

In Table I are given the renal blood flow, renal blood-pressure and urine excretion of an innervated kidney perfused by a heart-lung preparation, as well as the carotid blood-pressure of the kidney dog. For the sake of comparison these factors are also given before perfusion and after denervation. After evisceration and other preparations for the switch-over had been completed 0·65 to 0·53 c.c. of urine were being

TABLE I. Heart-lung preparation finished at 3.30. Perfusion of innervated kidney started at 4.24. Denervation of kidney at 4.59. 0·75 unit Parke Davis "Pitressin" into kidney circulation at 5.25. Kidney weight 38·0 g. Temperature of perfusion blood 37·5 to 38·0° C.

		Blood		Urine			
Time	Carotid blood-pressure	Flow c.c./min.	Pressure mm./Hg.	Flow c.c./10 min.	Cl. mg. p.c.	Absolute Cl.	
3^{44}–4^{08}	—	—	—	0·65	64	0·38	Fore period
4^{08}–4^{18}	97	—	—	0·53			
4^{24}–4^{38}	95	106	110	Slight	—	—	Innervated perfusion period
4^{38}–4^{58}	118	90	115	0·25	—	—	
5^{08}–5^{14}	—	175	100	8·6	64	5·50	Denervated perfusion period
5^{14}–5^{19}	—	185	102	12·4	44	5·45	
5^{19}–5^{34}	—	200	—	15·6	35	5·45	
5^{34}–5^{36}	—	142	102	2·0	—	—	Recovery from 0·75 unit "Pitressin" (Parke Davis)
5^{36}–5^{41}	—	145	100	3·0	340	10·2	
5^{41}–5^{46}	—	150	—	4·0	345	13·8	
5^{46}–5^{51}	—	160	98	5·2	200	10·4	
5^{51}–5^{56}	—	165	—	8·4	150	12·6	
5^{56}–6^{01}	—	167	—	12·8	140	17·9	

excreted per 10 minutes; the carotid blood-pressure being 97 mm. Hg. During perfusion with the renal nerve supply intact little or no excretion took place during the first 10 minutes, after which 0·25 c.c. per 10 minutes were excreted during the next 20 minute period; the renal perfusion pressure being 110 to 120 mm. Hg. and the renal blood flow 98 c.c. per minute. The kidney was then decapsulated and the adventitia removed from the artery. As this operation was in progress the urine flow began to increase to a notable extent, as did also the renal blood flow, so that within 20 minutes 15·6 c.c. of urine were being excreted per 10 minutes at a blood-pressure of 102 mm. of Hg. and a renal blood flow of 200 c.c. per minute. 0·75 of a unit of Parke Davis's "Pitressin" was then added to the circulating blood. The urine flow was at once inhibited, but gradually increased during the next 35 minutes to 12·8 c.c. per 10 minutes.

This experiment illustrates that until denervation is effected no polyuria is observable, but immediately after complete isolation of the kidney the rate of excretion rises steadily and at a rapid rate, until it may be as much as 30 times above that during the period of innervation; the blood flow is also noticeably increased. Another experiment illustrating these observations is given in Fig. 1. In this case after denervation, although the renal perfusion pressure dropped by nearly 20 mm. of Hg., the rate of excretion of urine was increased approximately six times. Similar results have been obtained in the ten experiments in which denervation was carried out.

The action of pituitrin upon the polyuria resulting from denervation

is similar in all respects to that described by Starling and Verney(4) for the heart-lung-kidney preparation. The behaviour of the chlorides in our experiments will be discussed in relation to this aspect of the problem later.

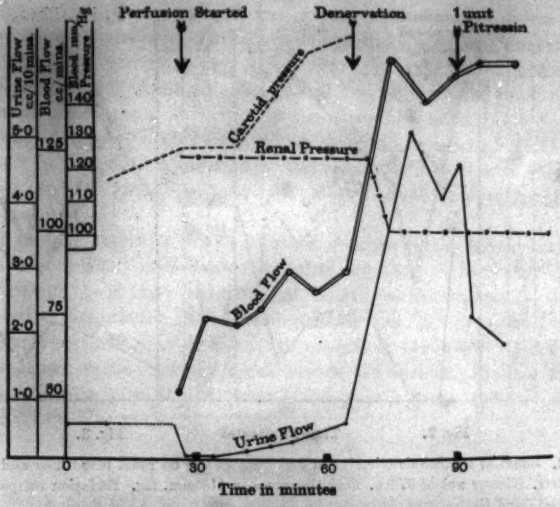

Fig. 1. Effect of denervation and "Pitressin" on renal blood flow and urine flow. Kidney weight 34·0 g. Perfusion temperature 38·0–39·0° C.

(b) Reflex alterations in the blood and urine flow of the innervated kidney perfused by the heart-lung preparation.

A steady urine flow was not always obtained during the period of perfusion in which the nerve supply to the kidney was undisturbed. In Figs. 1 and 2 the carotid blood-pressure of the kidney dog, the renal perfusion pressure and rate of blood flow, and urine excretion are compared. Fig. 1 shows that a gradual rise in the carotid pressure of the kidney dog takes place and is associated with a reflex increase in the renal blood flow and output of urine. The probable reason for this rise in blood-pressure in the kidney animal is that (as the result of inserting the aortic cannula for the renal perfusion) the circulation to the hind

limbs of the animal is occluded, and this gives rise to a reflex increase in the general blood-pressure of the animal. Another possible factor is the gradual leakage of blood from the heart-lung-kidney circuit, which was usually run at a higher pressure than that in the kidney animal. The

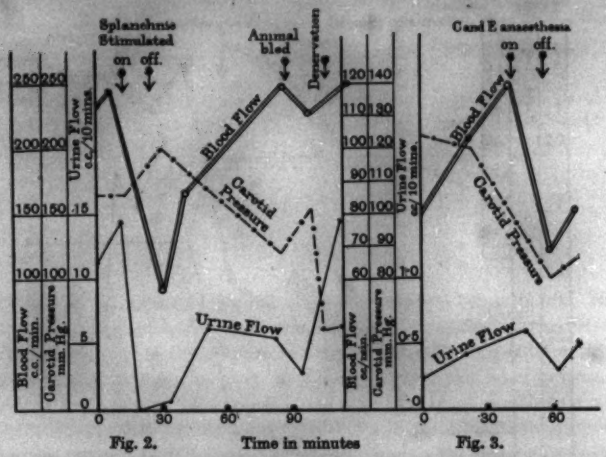

Fig. 2. Fig. 3.

Fig. 2. Effect of splanchnic stimulation and hæmorrhage on renal blood flow and urine flow. Kidney weight 57·0 g. Perfusion pressure 115 mm. Hg. Perfusion temperature 36·5–37·5° C.

Fig. 3. Effect of anæsthesia on renal blood flow and urine flow. Kidney weight 34·0 g. Perfusion pressure 115 mm. Hg. Perfusion temperature 37·0–38·5° C.

relationship between the blood-pressure of the kidney animal and the blood flow and urine output of its perfused kidney is, however, always destroyed by denervation of the kidney, when the typical polyuria already described occurs whether the animal is left alive or killed either by bleeding or anæsthesia.

In the second experiment (Fig. 3) the blood-pressure of the kidney animal was reduced by chloroform-ether anæsthesia. As can be readily observed this produced a reflex lowering of the renal blood flow and output of urine.

The series of experiments represented by Figs. 1 and 3 definitely indicate that alterations in the general blood-pressure of the kidney animal are capable of producing reflex alterations in the calibre of the

renal blood vessels, and that the rate of urine excretion follows the blood flow even when the renal blood-pressure remains constant.

Further reflex alterations in urine and blood flow are shown in Fig. 2. Appropriate faradic stimulation of the splanchnicus major inhibited the urine flow and markedly reduced the renal blood flow. This confirms earlier work on the whole animal. In the same experiment it is shown that excessive hæmorrhage of the kidney dog causes a similar reduction of renal blood flow and urine output, but that after denervation, although the blood-pressure of the animal was still low, an increased renal blood flow and urine output immediately occurred. The reflex inhibition of blood flow following hæmorrhage is very marked. In one experiment, as a result of extensive hæmorrhage, the renal blood flow was stopped entirely and the kidney became asphyxiated before denervation could be completed.

In general, therefore, a rise in blood-pressure in the kidney animal will produce reflexly an increased output of urine and an increased blood flow through the kidney. Reduction of the general blood-pressure, either by further anæsthesia or by hæmorrhage, has the opposite effect. Appropriate stimulation of the great splanchnic nerve will imitate the latter effect; section of the renal nerves the former. Similar reflex changes in the size of the renal blood vessels have recently been described by Heymans(6).

(*c*) *The excretion of chlorides.*

Urinary chlorides were estimated in several experiments. It was found that usually the amount present in the urine was low both during the fore-period and during perfusion with the renal nerves intact (50 to 100 mg. p.c.). After denervation, although the percentage output decreased concomitantly with the increased excretion of urine, the absolute rate of excretion remained practically constant. Upon reducing the denervation polyuria with commercial "pitressin," however, the absolute output was invariably increased two to three times. The addition of blood fresh from the kidney dog had a similar effect. In the one experiment during which the splanchnic nerve was stimulated such an increased absolute output of chlorides was not manifest, although the percentage amount increased with the decrease in urine flow which fell from 14·5 c.c. to an average of 3 c.c. per 10 minutes.

Discussion.

The above experiments are of interest, primarily because they indicate another factor which must be taken into account in interpreting the behaviour of the isolated kidney perfused by the heart-lung preparation. Starling and Verney(4) suggested that the characteristic increase of urine flow in such cases was due to a gradual disappearance of pituitrin or pituitrin-like substances from the circulating blood. In some of our experiments, however, we have perfused an innervated kidney for 50 minutes or more without obtaining any evidence of such a lack being evinced by an increased output of urine. Moreover, in all experiments, the heart-lung preparation was completed some time before shunting the kidney into the circuit; consequently, if the gradual disappearance of pre-existing post-pituitary principles is to be held responsible for the normal "ingravescent polyuria," such disappearance would, in our experiments, have been completed before the kidney was introduced into the circuit, and the polyuria should have been immediate. Actually there was no polyuria at all. Possible blood leakages from the animal into the heart-lung-kidney circuit were, in the majority of cases, prevented by keeping the renal blood-pressure higher than that of the kidney dog. If then simple denervation will increase the output of urine of the isolated kidney 30 times or more, it is extremely improbable that a further polyuria resulting from the absence of pituitary hormones could be ascertained in such a preparation.

In this connection it is worthy of note that part of the routine technique employed by Starling and Verney in the preparation of a heart-lung-kidney circuit is the addition of 1 to 5 g. of urea to the circulating blood for the purpose of counteracting the vasotonic effects of freshly defibrinated blood. We have, by adding similar amounts of urea to our preparations, obtained a marked diuresis (from 7·2 to 13·0 c.c. per 10 minutes). This practice is therefore, in our opinion, inadvisable, particularly since the same ends may be achieved by preparing the heart-lung preparation a few minutes earlier, and allowing the blood to circulate for a longer period.

The effect of innervation and denervation upon chloride excretion is not clear. In none of our experiments did we obtain an increase in the absolute output of chlorides as the result of a reflex reduction in the urine flow. Usually the absolute rate of excretion remained unchanged. Commercial pituitary extracts, however, invariably increased the absolute output. In this connection it is interesting that, in the protocols

given by Starling and Verney(4), the addition of pituitrin always resulted in an increased chloride output, whereas in the experiments of Verney(5), although the addition of a head to a heart-lung-kidney circuit reduced the output of urine, the absolute excretion of chlorides remained practically unaltered. We feel that this subject requires very careful investigation before alterations in the renal output of chlorides may be taken as evidence for or against the direct intervention of secretions of the pituitary in renal excretion, particularly after the unusual deviations in chloride output recorded in the whole animal by Magnus(3).

The reflex alterations of renal blood flow and urine excretion are of interest in suggesting a direct nervous control of renal function, apart from any changes which may follow directly upon changes in blood-pressure during hæmorrhage, shock, etc.

Summary and conclusions.

1. A method of perfusing an innervated kidney with a heart-lung preparation is described.

2. Such a preparation, unlike the isolated kidney perfused by the heart-lung preparation, shows no signs of excreting large amounts of hypotonic urine until denervation is effected.

3. With a constant perfusion pressure and blood composition a reflex rise in the renal blood flow produces an increase in urine flow.

4. Alterations in the blood-pressure of the kidney animal by hæmorrhage or shock produce reflex changes in the blood flow and urine output of the perfused kidney. When the blood-pressure is high the renal blood flow may be reflexly increased and the rate of urine excretion will then rise; and *vice versa*. Stimulation of the splanchnicus major (one experiment) of course reduced the blood flow and the excretion of urine, *i.e.* the same effects as were induced reflexly by a fall of blood-pressure in the kidney animal.

The expenses of this research were defrayed by a grant from the Royal Society to one of us (A.R.F.).

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FŒTAL RESPIRATORY REFLEXES[1].

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THE fœtus is usually considered to be in a state of respiratory quiescence before birth, and at birth the first inspiration occurs. This view is held by most physiologists since Zuntz(1, 2) who made the first systematic investigations of the fœtal respiration *in utero*. It is disputed by Ahlfeld(3) who stated on clinical grounds that it is possible to palpate the human fœtus and to feel respiratory movements occurring in the young child before birth. He considers these inspiratory and expiratory movements are incomplete only causing a to-and-fro movement of the amniotic fluid in the trachea. But as a result of this view he considers that there are shallow rhythmic movements occurring in intermittent intervals and that the fœtal respiratory centre is carrying on a rhythmic activity before birth. Birth is not in his opinion coincident with a sudden burst of activity on the part of the centre. The same type of movement was observed by Runge(4), but he hesitated to ascribe them to fœtal respiratory movements. The observations recorded in this paper do not lend support to Ahlfeld's view.

The term "fœtal apnœa" is frequently used to describe this condition of respiratory quiescence. Some physiologists object to the term on the grounds that it implies a previous condition of respiratory activity. On the grounds of convenience it has been decided to continue its use in this paper with the defined meaning of inactivity of the respiratory reflex, and without any implication of a previous state of active respiratory movement.

Assuming therefore that fœtal apnœa is a real normal physiological state, its cessation may be due to a number of possible causes. Cohnstein and Zuntz(5) working on the fœtal lamb observed that clamping the umbilical cord caused respiration. They considered that insufficient

[1] A portion of a thesis approved for degree of Doctor of Science in the University of London.

venosity was the cause of apnœa. Pflüger(6) thought that for the fœtus of the dog interruption of the placental circulation was inadequate without cutaneous stimulation by cold air. Preyer(7) maintained that cutaneous stimuli are essential whether or not the umbilical cord was clamped.

The possible causes of fœtal apnœa include:

(i) Incomplete development of the respiratory centre or centres.

(ii) Complete development with inadequate stimulation. This may be due to: (*a*) Abnormally high threshold value compared with the adult centre. (*b*) Normal threshold but subliminal stimulus.

(iii) Complete development and normal stimulus but inhibition of the centre by some inhibiting factor.

This paper records the results of investigating some of the effects of the nervous system on the respiratory mechanism.

Methods.

The animals used were pregnant goats one to two months before full term. The reason for this was that at full term it was found that the fœtus could easily be stimulated to respiratory efforts but could not easily be stopped, whereas the premature fœtus after being stimulated to respiratory movements stopped spontaneously and easily on removal of the stimulus. The technique was the same as previously described(8), the fœtus after Cæsarean section being immersed beneath 0·85 p.c. saline at 38° C. on a wire frame to keep it steady and fixed.

Respiratory movements were noted by (*a*) direct observations; (*b*) kymograph records.

The records were made either by a clip attached to the skin at the xiphisternum or by a cannula in the fœtal trachea containing saline, so that inspiration did not lead to the entry of air into the lungs. The tubing from the cannula to the recording tambour contained air, but this did not reach to the trachea.

Experimental investigations.

(i) *Manipulation.* Lifting the fœtus from the uterus and placing on a supporting wire frame sometimes caused fœtal respiratory movements which were slow and laboured and stopped when manipulation ceased (Figs. 1, 3, 4 *b*, 4 *c*, and 4 *d*). They were less frequent in rate the less the development of the fœtus.

(ii) *Section of the skin.* Clean skin incisions with a scalpel did not

cause fœtal inspiratory movements whether the mother was anæsthetized or decerebrated.

Fig. 1. Goat. Urethane. Fœtal respiration. Respiratory movements while manipulating fœtus.

Fig. 2 a. Goat. Urethane. Fœtal respiration. Respiratory movements due to sciatic stimulation.

Fig. 2 b. Goat. Urethane. Fœtal respiration. Respiratory movements due to sciatic stimulation.

Fig. 3. Goat. Decerebrated. Fœtal respiration. Respiration initiated by manipulation, increased by sciatic stimulation. Shows respiratory after-discharge.

(iii) *Afferent nerve stimulation—Sciatic.* Exposure of the sciatic nerve in the leg by incision through the skin and leg muscles did not cause any

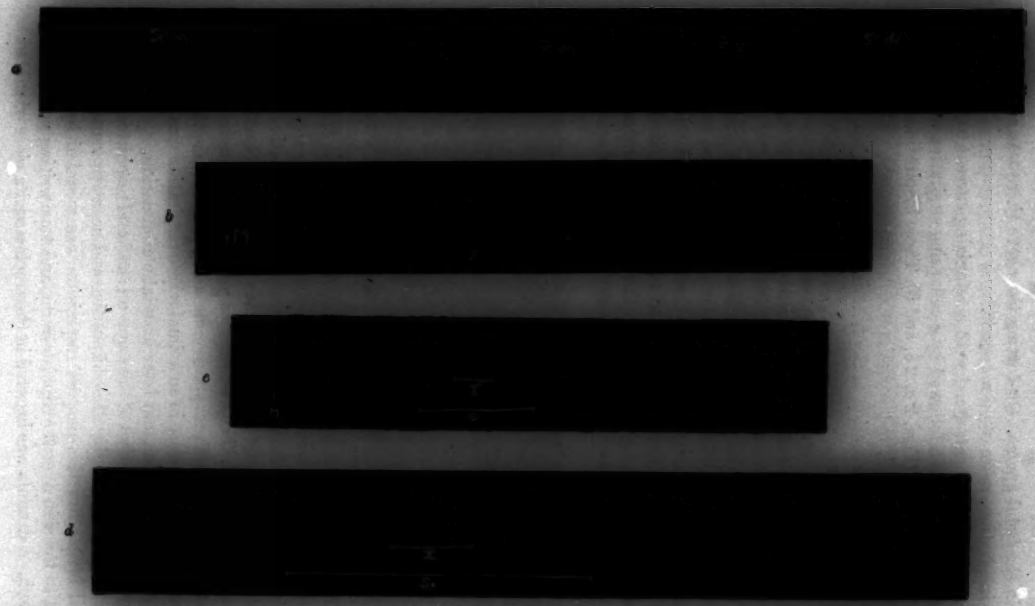

Fig. 4 *a*. Goat. Urethane. Fœtal respiration. Respiration due to sciatic stimulation inhibited by stimulating central end of left vagus.

Fig. 4 *b*. Goat. Decerebrated. Fœtal respiration. Respiration due to manipulating fœtus inhibited by weak stimulation of left vagus nerve. Shows a slight latent period.

Fig. 4 *c*. Similar to Fig. 4 *b*.

Fig. 4 *d*. Similar to Figs. 4 *b* and 4 *c*. Respirations initiated by manipulation. Vagus effect partly counteracted by sciatic stimulus.

change in the fœtal apnœa. On cutting the nerve there was sometimes a single inspiration, more often no result.

Stimulation of the central end with a tetanizing current caused slow irregular inspirations each followed by a collapsing expiration at the rate of 12 to 15 per minute (Figs. 2 *a*, 2 *b*, and 3). These inspirations stopped when stimulation ceased, but sometimes not immediately, going on for a varying period after cessation of the tetanizing current. That is to say, they might show an after-discharge. This effect is shown in Figs. 3 and 4 *c*. It was repeatable whenever required. The movements when they ceased, stopped completely. This effect was obtained by stimulation of the sensory nerves in the fore-limb as well as in the hind-limb.

The effects of afferent nerve stimulation in the fœtus and mother were compared. The minimal electrical stimulus to produce respiratory movements in the fœtus was stronger than the minimal stimulus to increase respiration in the mother. These are shown by the following figures obtained from two goats. They give the maximal distances between the primary and secondary coils for the maternal and fœtal sciatic nerve stimulations.

Fœtal	Maternal
5·5	9·0
6·7	10·5

The strengths of stimuli are inversely proportional to these figures.

(iv) *Vagal stimulation.* Cutting one or both vagi had no effect on fœtal apnœa. Stimulation of the peripheral end of one cut vagus with a tetanizing current caused slowing of the heart beat, after a latent period of 2 to 3 seconds. Stimulation of the central end had no effect on the apnœa, but if fœtal respiratory movements were caused by faradizing the central end of the sciatic nerve or by manipulation of the fœtus, then these respiratory movements could be inhibited by simultaneous stimulation of the vagus. This inhibition varied according to the relative strength of stimulation of the sciatic and vagus nerves respectively. Instances are shown in Figs. 4 *a* to 4 *d*. The first shows a perfect vagal apnœa imposed on a sciatic dyspnœa which reappears on ceasing to stimulate the vagus. The second was a similar case in an animal where respiration was initiated primarily by manipulation and later by sciatic stimulation.

In investigating the effect of the vagus on the fœtal respiratory centre, recourse was also had to the effect of positive and negative ventilation of the fœtal lungs. For this purpose respiration was recorded by a hook attached to the skin over the xiphisternum, and to a writing point on a kymograph. A cannula was inserted in the trachea and a piece of rubber

tubing attached to it. The whole was filled with saline. The tubing from the trachea and cannula was blown down or evacuated by a pump. The respiratory record was made at the same time.

The effect of increasing the intratracheal pressure is shown in Fig. 5.

Fig. 5. Goat. Urethane. Fœtal respiration. Respiration recorded by xiphisternal attachment. Effect of prolonged positive pressure in trachea, giving a single inspiration. Small waves record maternal dyspnœa causing movements of saline of bath and of fœtal body immersed in it.

The result is a few irregular inspirations, but no expiratory tetanus as described by Head (9). On the other hand, the effect of a steadily maintained negative intratracheal pressure is to call forth a few inspirations but no inspiratory tetanus (Fig. 6).

Fig. 6. Goat. Urethane. Fœtal respiration. Respiration recorded by xiphisternal attachment. Effect of prolonged negative pressure in the trachea, yielding a single big inspiration. Small waves as in Fig. 5.

(v) *The fore-brain.* The above results show that the fœtal respiratory centre can respond to normal stimuli, but it is unable to do so unless they are very strong. The cause of this inertia on the part of the centre is a matter for investigation. It is not due to cerebral inhibitory influence, since decerebration of the fœtus does not cause apnœa to stop and normal respiration to supervene. In one case in which this was done rapid shallow respiration followed, which soon stopped, the respirations becoming more and more infrequent. They are shown in Fig. 7. In two other cases no respiration followed decerebration, the animal maintaining its apnœa. On stimulation of the sciatic nerve in the decerebrate fœtus

Fig. 7. Goat. Urethane. Foetal respiration. Respiration recorded by intratracheal cannula. Shows (1) Effect of decerebrating foetus (Dec.). Gives quick shallow respirations going slower afterwards. (2) Sciatic stimulation (signal) giving slightly quicker and much deeper respirations (Sc.). (3) Post-sciatic stimulation after-discharge. (4) Manipulation gasps (M.). (5) Foetal spaces. (Second stimulation signal is not a stimulation of the nerve.)

the normal sciatic dyspnœa supervened. This was followed in Fig. 7 by a gasping type of respiration.

Discussion.

The experiments described in this paper demonstrate that the central nervous system of the fœtal goat is sufficiently developed at 4 to 6 weeks before full term to respond to strong afferent nervous stimuli. The nature of the response depends on the nerve stimulated, a nerve which excites respiratory movements in the adult excites them in the fœtus, while one such as the vagus which inhibits in the adult, inhibits in the fœtus. The quality of the respiratory response differs however. In the premature fœtus the response is irregular and laboured and does not exhibit the regular rhythmicity seen in the adult. The strength of stimulus required to produce the response is greater in the fœtus than the strength required to modify the normal respiration of the maternal animal. It is inferred consequently that the respiratory central mechanism has a higher threshold value for afferent nerve stimulation in the fœtus than in the adult. This reflex response to afferent nerve stimulation exhibits after-discharge. The response to afferent nerve stimulation always ceases when the periods of stimulation and after-discharge have ceased, but the latter period may be prolonged as in Fig. 4 *b*. This prolongation appears to be related to the degree of development, being more prolonged the nearer to full term the fœtus is.

Always therefore in the premature fœtus, but not necessarily in the full-term fœtus, we obtain a return to the normal fœtal condition of respiratory quiescence following afferent nerve stimulation.

The causation of this high threshold value is of interest, but beyond saying that it is not due to inhibitory stimuli from the cerebrum (a possible but improbable cause) it is difficult to ascribe a definite reason for it.

Similarly it is impossible to give an explanation of fœtal apnœa based on these experiments, beyond pointing out the existence of this high threshold to afferent stimulation and the further fact that the normal afferent stimuli *in utero* are subliminal. The consideration of the chemical factors controlling the respiratory reflex in the fœtus is deferred.

Summary.

1. The fœtal respiratory centre responds to the same stimuli which affect the adult respiratory central mechanism, but the strength of stimulus needs to be stronger than the normal strength for the adult.

2. The response to an exciting stimulus is slow, laboured and irregular, and does not exhibit the same rhythmicity as the adult reflex.

3. The reflex response exhibits after-discharge, but when this after-discharge ceases the fœtus returns to the normal fœtal condition of respiratory quiescence.

4. The centre being excited to reflex activity an inhibitory (vagal) stimulus depresses the activity, but the result is the algebraic sum of the two opposing stimuli.

5. It is inferred that the fœtal respiratory centre exhibits a higher threshold value to afferent nerve stimulation than the adult centre. This is not due to inhibiting stimuli from the cerebrum.

In conclusion I would take this opportunity of thanking Prof. J. Barcroft, F.R.S., for helpful criticisms of this work.

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THE ACTION OF CAFFEINE ON THE ISOLATED KIDNEY OF THE DOG.

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Introduction.

The diuretic action of caffeine has been attributed to a great variety of causes. Among extra-renal factors, a nervous mechanism has received little attention since it was shown by von Schroeder(1), Frey(2) and others that denervation of a kidney promotes rather than lessens the response to caffeine. A chemical mechanism has, however, recently been advanced, which depends on a reduction by caffeine of the affinity of plasma proteins for water, and consequent acceleration of a filtration process in the glomeruli; this is supported by the experiments of Ellinger, Heymann and Klein(3), Veil and Spiro(4) and Szelöczey(5). Among hypotheses involving a local action of caffeine on the kidney, that of a stimulant action on the secretory epithelium was advocated by von Schroeder(1) as a consequence of a lack of parallelism between the observed diuretic and the inferred vascular changes in his experiments on the rabbit. Richards and Plant(6) produced caffeine diuresis without increase of blood flow in the rabbit's kidney, and Schmidt(7) and Hartwich(8) produced caffeine diuresis without increase of perfusion flow in frogs' isolated kidneys perfused with Ringer's solution.

Cushny and Lambie(9), in commenting on the lack of parallelism between the blood flow and urine flow responses of the rabbit's kidney to the caffeine stimulus, state: "The simplest view which reconciles the observations is that caffeine causes diuresis by reducing the resistance to filtration through the glomerular capsule by a specific action on its cells."

The secretory hypothesis derived further support from Barcroft and Straub(10), who found that caffeine increased the oxygen consumption of the kidney of cats and rabbits, though their interpretation seems not unequivocal, since Fee and Hemingway(11) showed that a rise of arterial pressure would increase the oxygen consumption of the dog's

kidney, and Hayman and Schmidt(12) detected no influence of caffeine on the metabolism of a similar organ.

The intimate implication of the hypothesis associating caffeine diuresis with an action on the parenchyma, as distinct from the vasculature of the kidney, may be (i) stimulation of secretion of urine by the tubular epithelium, advocated by Barcroft and Straub(10); (ii) inhibition of reabsorption in the tubules, advocated by Sobieranski(13) and Tashiro(14) as a result of somewhat ambiguous experiments on excretion of dyes, and opposed by Masuda(15) owing to the persistence of caffeine action in the presence of HCN; and (iii) increased permeability of the glomerular membrane, widely favoured(9) in the absence of contrary indications.

Loewi, Fletcher and Henderson(16) opposed the secretory hypothesis of von Schroeder on the grounds that the diuretic action of caffeine could be adequately explained by an hypothesis according to which renal vaso-dilatation was the primary and essential effect of the drug. Phillips and Bradford(17) had shown that caffeine usually increased the volume of the kidney, and Loewi never observed diuresis in the rabbit without concomitant dilatation, as indicated by changes of colour of the blood flowing in the renal vein. He noticed also the occasional absence of increase in volume of the kidney which had been interpreted as an absence of vascular response to caffeine, but found that in these, as in cases when the volume of the kidney was kept constant by a plaster jacket, there was the same evidence of an increased blood flow through the organ. Although the close association of vaso-dilatation and caffeine-diuresis is confirmed by our experiments on the isolated kidney of the dog, the direct causal relationship claimed by Loewi is unlikely, in view of experiments on the same preparation showing the essential independence of the rates of perfusion flow and urine flow (Dreyer and Verney(18)). In any attempt to determine the mechanism of the action of caffeine, the consideration of two further vascular factors is demanded by the work of Richards and his co-workers. First, evidence has been brought forward by Richards and Plant(19) and Mendenhall, Taylor and Richards(20), showing that in the rabbit's kidney the vasa afferentia and the vasa efferentia may be differentially constricted by small doses of certain drugs; if, then, caffeine induced relatively greater dilatation of the former, an increased glomerular capillary pressure, together with an increased blood flow, would ensue. Second, direct observations of the circulation through the glomeruli in the frog's kidney by Richards and Schmidt(21) have shown that both the num-

ber of active glomeruli, and the degree of activity of these, are widely variable under experimental conditions, and are increased by caffeine; consequently caffeine increases the glomerular capillary surface.

The work of Richards and his colleagues further suggests a possible interpretation, in terms of an action by caffeine on the vasculature of the kidney, of such a degree of independence of the diuretic and blood flow responses to caffeine as was encountered by Cushny and Lambie. Cushny and Lambie state: "This acceleration of the blood flow appears at first sight to give an adequate explanation of the diuresis. But in the experiment from which Fig. 4 is drawn the increase in the urine began rather before the accelerated flow and in all cases the diuresis continued long after the blood flow had returned to the normal rate or was even slower." The passing and inversion of the increase in blood flow may be determined by a more rapid recovery by the vasa efferentia than by the vasa afferentia from the dilator effect of caffeine, and by a progression of this recovery into a supernormal state of vascular tone. Von Schroeder showed in the case of the rabbit that the total solids in the urine excreted per unit of time increased in caffeine diuresis, but not to the same degree as the water. And more recent work has demonstrated an approach, during caffeine diuresis, in the composition of the urine towards that of the plasma. It will be evident, from the work to which reference has been made, that no insuperable difficulties have yet been encountered to an interpretation of the responses of the kidney to caffeine in terms of an action on the vasculature of the glomeruli.

The following experiments were performed in order to supply some of the data, which will ultimately enable discrimination between these hypotheses to be justified. In performing them we have had two main objectives in view: first, to determine the responses of the isolated kidney in so far as they are reflected in changes in the rates of blood and of urine flow and in the composition of the urine, to the presence of caffeine in the blood stream; and second, to make a quantitative comparison between these responses and those elicited by a simple rise in perfusion pressure.

Method.

The actions of caffeine on the isolated kidney have been investigated in two ways; first, by comparing successive changes in the same organ; and second, by comparing simultaneous changes in a pair of kidneys, one of which serves as a control. In the first class of experiments, on the single heart-lung-kidney, the technique adopted was the same as that

described by Starling and Verney(22), except that greater precautions were taken to prevent dilution of the defibrinated blood with saline. In a few of the later experiments of this group, the more compact heart-lung apparatus described(23, 24) in connection with the heart-lung-double-kidney preparation was used. The technique of the second class of experiments on the double heart-lung-kidney preparation is described briefly elsewhere(25). Urea, usually 1 g., was added to the heart-lung circulation before the kidneys were connected to it. In the earlier experiments on the single heart-lung-kidney preparation, 1 g. of glucose was added at the same time. In the earlier experiments caffeine citrate, and in the later ones the base itself, was used. The drug was administered by adding a solution in saline to the venous reservoir. The volume of circulating blood was approximately 700 c.c. and the dosage of drug, except in the experiments described in section (4), varied in different experiments between 50 mg. of the base and 250 mg. of the salt. These were all supramaximal doses, the maximal concentration of base in this preparation being in the region of 1 : 25000.

Urea was estimated by the urease method, chloride expressed as NaCl, by the method of Millard Smith(26). In all the figures the time of a plotted point is the mid-time of the interval during which the sample of urine was collected.

The temperature of the blood in the renal cannulæ ranged in different experiments from 36·0° C. to 38·5° C., but its variation in any one experiment was usually less than 0·5° C.

Experimental.

1. *The influence of caffeine on certain properties of the isolated kidney.* The most constant change which caffeine in large doses effects in an isolated kidney is an increase in the rate of blood flow through the organ (Figs. 1–7). In successful experiments on forty kidneys the effect was well marked in all but those in which the rates of blood flow were varying too rapidly to make a change in them significant. Diuresis results from caffeine administration (Figs. 1–7) under rather more restricted a group of experimental conditions. Six of our series of kidneys failed to respond in this way; that the lack of response in these was abnormal was suggested by apparent fibrosis in several of them. A few others showed only a small or indefinite diuretic response. A noteworthy difference between the kidneys which yielded a well-marked diuretic response to caffeine, and those which did not, was found by comparing the rates of perfusion flow before administration of the drug. Twenty-four kidneys which were

sensitive to caffeine were being perfused at a rate of 121 ± 26 c.c. per min., whereas eleven kidneys which were insensitive were being perfused

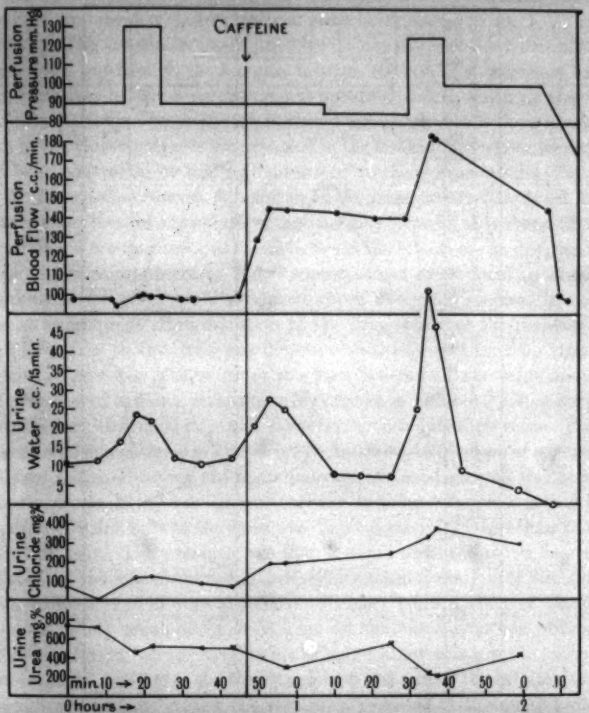

Fig. 1. Single heart-lung-kidney preparation. Comparison of the effects of increased perfusion pressure, and caffeine citrate (0·20 g.), applied consecutively to the same kidney. The stimuli were arranged to induce about equal increases in the rate of urine secreted by the kidney.

at 197 ± 53 c.c. per min. Although these standard deviations of the mean rates of perfusion are relatively large, owing in part to variations of perfusion pressure and the size of the kidneys in the different experiments, it is evident that the blood flow through the insensitive kidneys was considerably greater than that through the sensitive organs. This relation

between the insensitivity to caffeine and the relatively large perfusion flow could be simply explained by supposing that an unusually low

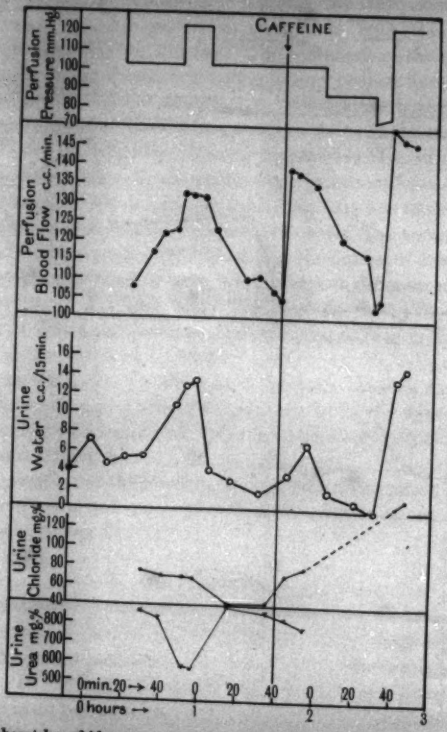

Fig. 2. Single heart-lung-kidney preparation. Comparison of the effects of increased perfusion pressure, and caffeine citrate (0·20 g.), applied consecutively to the same kidney. The stimuli were arranged to induce about equal increases in the rate of blood flow through the kidney.

degree of tone in the glomerular vessels accounted for both. In this connection it is interesting to note that small doses of adrenaline, such as produce a concentration in the heart-lung-kidney circulation of one to three in ten million, effect an increase in urine secretion accompanied by a reduction in the rate of perfusion flow, at constant perfusion pressure.

Larger doses reduce or arrest the secretion, and produce a much greater diminution in the perfusion flow.

The concentration of urea in the urine is lowered by caffeine, but the absolute amount secreted in a given time is increased (Figs. 1, 2, 4, 5, Table I). The concentration of chlorides is usually increased by caffeine (Figs. 1–7), but though the increase is often 100 p.c. or more, there have been instances in which no change has occurred and in which its absence could be attributed to no known factor. Caffeine produced these changes both in the thirty polyuric kidneys and in the ten kidneys whose secretion had been controlled by adding "pituitrin" to the perfusion fluid (Fig. 3).

2. *The relation between the changes in the urine flow and the blood flow.* As a first step toward an analysis of the relations between the several effects of caffeine, a comparison was made between the effects of the drug and of a rise in perfusion pressure. Fig. 1 represents an experiment in which a temporary rise of pressure produced about the same increase in urine flow as subsequent administration of the drug, whereas the response of the blood flow to the drug was disproportionately greater than that to the rise of pressure. The pressure was then lowered till the urine flow, in the presence of caffeine, was rather lower than it had been before its administration; the blood flow still, however, retained its high value. Fig. 2 represents a complementary experiment, in which an increase of perfusion pressure achieved about the same increase of blood flow as was subsequently produced by addition of caffeine to the perfusate, whereas the response of urine flow to the drug was disproportionately less than to the rise of pressure. The pressure was then lowered until the blood flow was reduced to the value obtaining just before administration of the drug, when urine secretion was abolished. Further confirmation of the disproportionately great effect of caffeine on the blood flow was obtained from experiments on the double heart-lung-kidney preparation, such as that recorded in Fig. 4. In these, caffeine was added to the circulation through one kidney, whilst the perfusion pressure of the other kidney was raised to values which kept equal the rates of urine secretion by the two kidneys. The organ under the influence of the drug maintained a blood flow greater than that of its control at the higher pressure throughout the rest of the experiment. Such experiments on the double heart-lung-kidney preparation are summarized in Table I. It is evident, therefore, that a supramaximal dose of caffeine changes the relation, in the isolated kidney, between the rate of urine secretion and the rate of blood passing through the organ, and that the increase of blood flow is both the more consistent, and relatively the greater, with respect to the changes

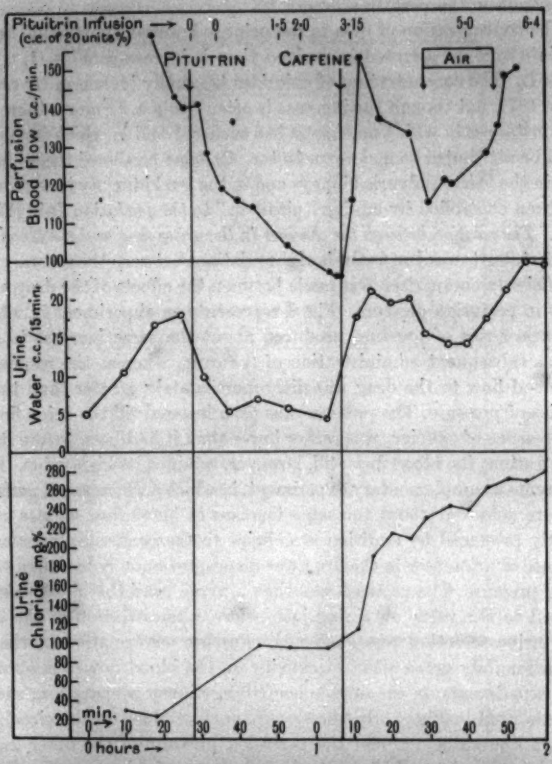

Fig. 3. Single heart-lung-kidney preparation. The action of caffeine citrate (0·20 g.) on a kidney under the influence of post-pituitary extract. Owing to unusual insensitivity of the kidney, as much as 4 units of "pituitrin" were added at "28 minutes," and its concentration in the circulating fluid was maintained by constant infusion of a solution containing 20 units per 100 c.c. of saline, from a burette, the progressive readings of which are shown at the top of the figure. During the period marked "air," the usual respiratory mixture of 5 p.c. carbon dioxide in oxygen was replaced by air. The effects of acapnia here illustrated have been repeatedly observed and they may be interpreted qualitatively in terms of a fall in the effectiveness of the pressure stimulus.

associated with increase of perfusion pressure. Gremels(27) inferred from experiments on the dog's isolated kidney that caffeine had a relatively unimportant and inconsistent action on the blood flow.

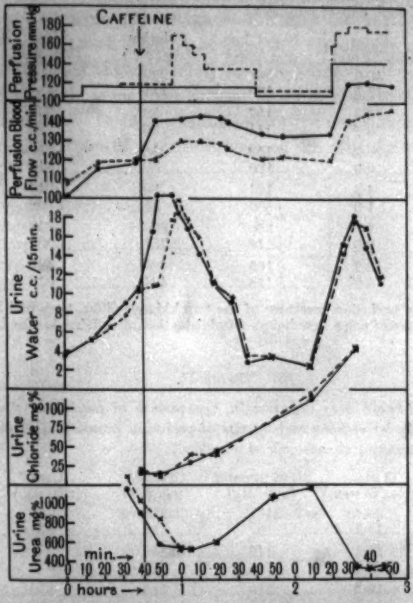

Fig. 4. Double heart-lung-kidney preparation. Comparison of the action of increased perfusion pressure in one kidney with that of caffeine (0·05 g.) applied simultaneously to the other. Caffeine was administered to the left kidney (continuous lines), and the pressure stimulus, applied to the right (broken lines), was adjusted to secure about equal rates of urine secretion by the two kidneys.

3. *Comparison of the effects of caffeine, and of increase of perfusion pressure, on the composition of the urine.* The constituents of the urine chosen for analysis were urea and chloride, on account of the theoretical interest attaching to changes in their concentration. From experiments on the single heart-lung-kidney preparation, it was evident that the increase in rate of urine flow produced by caffeine was accompanied by changes in composition qualitatively the same as those by which an increase, produced by a rise in perfusion pressure, was accompanied. The

TABLE I.

Double heart-lung-kidney experiments. Comparison of conditions when one kidney is under the influence of caffeine, and the other is a control. (Effects of caffeine represented by the upper of each pair of numbers.)

Exp.	Urine (c.c./15 min.)	Blood flow (c.c./min.)	Chloride (mg. p.c.)	Urea (mg. p.c.)	Blood pressure (mm. Hg)
56	40·0	288	280	—	160
	12·4	150	144	—	160
58	16·3	157	50	770	119
	8·7	111	36	1220	119
60	20·3	140	16	580	117
	10·9	120	10	840	119
61	4·3	104	50	1940	106*
	1·6	103	34	3100	110
62	9·7	250	850	830	121*
	8·0	214	756	960	113
63	16·2	163	400	485	108
	5·9	148	464	920	108

* Where the perfusion pressures of the two kidneys differ, they had been adjusted to secure equal rates of urine flow before administration of caffeine to one kidney.

TABLE II.

Double heart-lung-kidney experiments. Comparison of conditions when urine flow is increased equally by caffeine and by rise of perfusion pressure. (Effects of caffeine represented by the upper of each pair of numbers.)

Exp.	Urine (c.c./15 min.)	Blood pressure (mm. Hg)	Chloride (mg. p.c.)	Urea (mg. p.c.)	Blood flow (c.c./min.)
56 (*a*)	10·4	107	128	—	178
	11·7	107	118	—	125
56 (*b*)	23·1	146	350	—	240
	22·6	206	346	—	176
58 (*a*)	16·2	119	110	570	159
	16·6	156	118	490	120
58 (*b*)	3·5	119	438	670	163
	3·6	129	312	760	126
60 (*a*)	11·1	117	46	600	142
	11·2	135	40	620	128
60 (*b*)	2·6	108	112	1170	132
	2·6	113	120	1180	120
60 (*c*)	17·7	142	180	340	160
	18·3	175	180	330	141
61	3·5	97	520	1230	97*
	3·5	128	450	1320	115
62	9·3	121	870	780	257
	9·2	120	770	790	220
63	12·7	99	300	440	170*
	12·7	124	390	475	176

* These values appear atypical only in a table which omits description of previous differences between each of the pair of kidneys.

double heart-lung-kidney preparation(24) afforded a method by which such changes could be accurately compared at equal rates of urine flow. The two circulations were separated after approximate equality of secretion by the two kidneys had been reached. Caffeine was added to one circulation, and after a short interval during which its effects could be observed, the perfusion pressure of the other circulation was raised so as to equalize the rates of secretion of the two kidneys. Pressure adjustments were then made from time to time as seemed necessary to match the rates of urine flow. In such circumstances the concentrations of urea were equal when the rates of urine flow were equal. Fig. 5 represents such

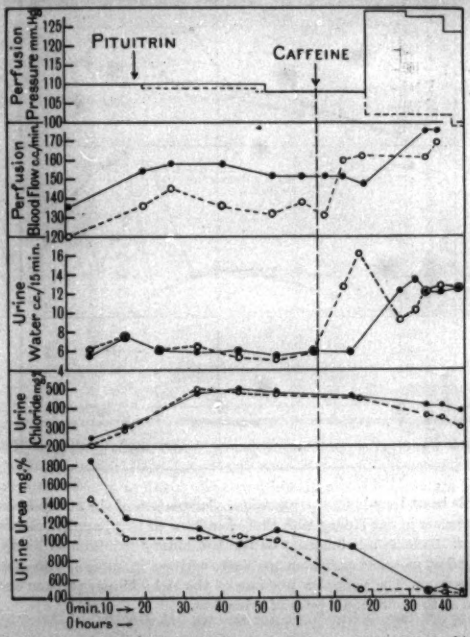

Fig. 5. Double heart-lung-kidney preparation. Comparison of the action of increased perfusion pressure in one kidney with that of caffeine (0·05 g.) applied simultaneously to the other, in the presence of post-pituitary extract (1 unit added to each circulation at "18 minutes"). Caffeine was added to the circulation through the right kidney (broken lines), and the pressure stimulus applied to the left (continuous lines), and so adjusted as to secure approximately equal rates of urine secretion by the two kidneys.

an experiment, showing the equality of urea concentration in urine when the rates of secretion of a pair of kidneys are kept equal. These, and other experiments of the same kind, are summarized in Table II. The results

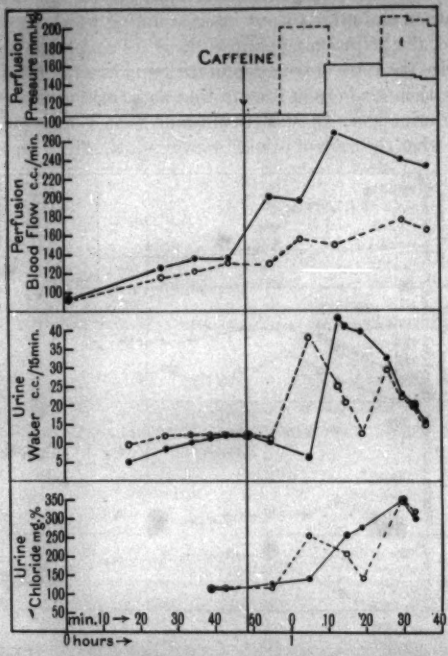

Fig. 6. Double heart-lung-kidney preparation. Comparison of the action of increased perfusion pressure in one kidney with that of caffeine (0·15 g.) applied simultaneously to the other. Caffeine administered to the left kidney (continuous lines) induced no diuretic effect at a low perfusion pressure, whereas it induced a marked effect at a higher pressure. The perfusion pressure of the right kidney (broken lines) was then raised so as to secure equal rates of urine flow.

of chloride estimations are not quite so unequivocal, since wider variations in their values may occur even when the urines from a pair of kidneys under control conditions are compared. Within the limits of accuracy set by this difficulty, it would seem that the same conclusion holds, namely, that the concentrations of chloride are equal in urines

from a pair of kidneys, when their rates of secretion are equal, and when one is under the influence of caffeine and the other submitted to the higher perfusion pressure. Fig. 6 illustrates this relation. The values for chloride concentrations in Table II show that the differences between those for caffeine diuresis and pressor diuresis are fairly small, and random in direction.

It would seem legitimate to extend this identity to any remaining solutes in the urines at equal rates of flow, and to conclude that in so far

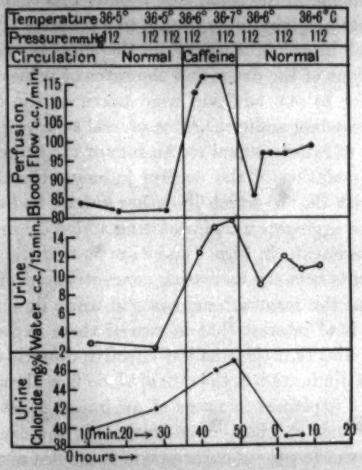

Fig. 7. Double heart-lung single-kidney preparation. Changes in a single kidney perfused in turn with (i) a normal heart-lung circulation, (ii) a heart-lung circulation containing caffeine citrate (0·10 g. in about 750 c.c. of blood), and (iii) again the normal heart-lung circulation. The degree of reversibility of the effects is shown.

as its effects on the rate and composition of the urine are concerned, caffeine exerts its action by increasing the effectiveness of the pressure stimulus.

4. *The relation between the concentration of caffeine in the blood and the magnitude of the response of the kidney.* We have performed several experiments on the double heart-lung-kidney preparation in an attempt to discover whether any relationship could be identified between the concentration of caffeine in the blood stream and its effects on the rates of blood and of urine flow. That the response of the kidney is not of the

"all or none" variety, is apparent from experiments designed to test the applicability of the method to the investigations for which it was conceived (34), see Fig. 5 of that paper). In the experiments which we now propose to describe, increasing doses of the drug have been added to one circulation, and their effects determined quantitatively by reference to the rate of urine and of blood flow of the other kidney which is functioning throughout as a simultaneous control. It had already been found that the blood flow and urine flow increments in response to the addition of a single dose of caffeine to the blood rose rapidly and then more slowly to reach their maintained maximum from 5 to 9 minutes after the addition of the drug. Such intervals of time were therefore allowed to elapse between the additions of the drug; and the rates of urine and blood flows immediately prior to one addition were taken as the response of the kidney to the preceding addition. After several such responses had been obtained, a dose of caffeine equal to the sum of the successive doses was added to the circulation of the control kidney. Usually its response agreed closely with that to which the other kidney had attained, as the result of the same aggregate amount of drug added during the preceding stages of the experiment. In Figs. 8 and 9 are plotted the relations found in four experiments between increasing concentrations of caffeine in the blood stream and the resultant increases in urine flow and blood flow respectively. It is of interest that in two of these experiments (nos. 1 and 4) there is a hint that the smallest effective diuretic dose of caffeine may give rise to a diminution in the rate of blood flow through the kidney, and this might be explained in terms of an initial constrictor response by the vasa efferentia to small concentrations of the drug. In Fig. 8 it will be seen that in the case of experiments 3 and 4 a distinct falling off in the urine flow response is observed at high concentrations of caffeine in the blood stream, but that this is due to the intrusion of some uncontrolled factor would seem probable from the fact that, in these two experiments, the addition of the aggregate dose to the control heart-lung-kidney gave a very much larger response than that given by the other kidney to the same dose spread over an interval of time. It would seem that graphs 1 and 2 should be taken as representing a more accurate picture of the relation between the concentration of caffeine in the blood stream and the urine flow response by the kidney, the initial part only of the response being given by graph 1.

5. *The reversibility of the action of caffeine.* Two experiments performed on a double heart-lung single-kidney preparation indicated that the actions were partly reversible when caffeine was administered in the

concentrations obtaining in these experiments. Fig. 7 presents the results of one such experiment. Blood from the two circulations was

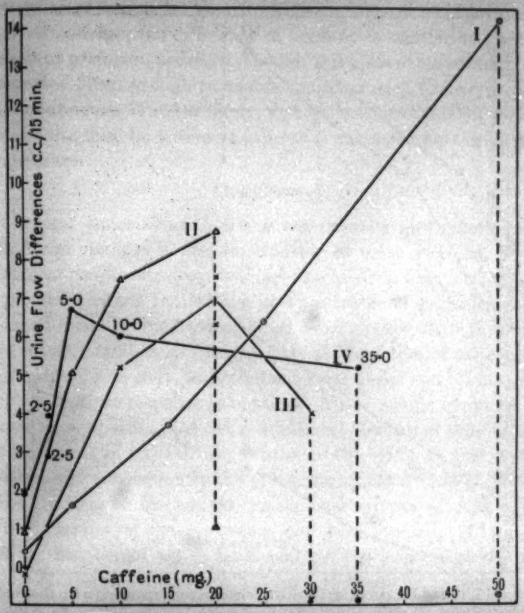

Fig. 8. Curves I, II and III show (for three experiments) the relation between the doses of caffeine present in one circulation of a double heart-lung-kidney preparation, and the differences between the rates of urine secreted by the affected and the control kidneys. Curve IV represents an experiment on a similar preparation, in which caffeine was added in unequal doses to the circulations through the respective kidneys; the doses in the less concentrated circulation are the figures near the dots on the curve, while the abscissæ represent the differences in dosage between the two circulations. The concentration curves extend only to the vertical interrupted lines. The lowest points on these lines represent the differences found between the kidneys when, towards the end of the experiment, they were being perfused with blood of the same composition, obtained by the thorough mixing of the two circulations. This was brought about by allowing the blood passing through the peripheral resistance of the one circulation to return to the venous reservoir of the other, and so to "cross-circulate."

allowed to mix until just before addition of 0·10 g. caffeine citrate to the reservoir on one side, when there were probably about 750 c.c. fluid in each circulation. After the effects had been observed, the kidney was

switched over to the caffeine-free circulation, and about 100 c.c. of blood from the kidney separately collected in order to remove as much caffeine

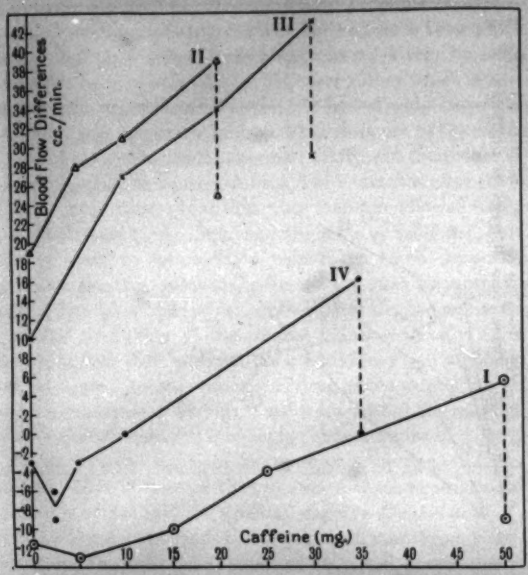

Fig. 9. Curves I, II and III show (for three experiments corresponding with those of Fig. 8) the relation between the doses of caffeine present in one circulation of a double heart-lung-kidney preparation, and the differences between the rates of perfusion flow through the affected and control kidneys. Curve IV represents a similar experiment, in which caffeine was added in unequal doses to the circulations through the respective kidneys; the doses in the less concentrated circulation are the same as those shown on the corresponding curve in Fig. 8, while the abscissæ represent the differences in dosage between the two circulations. The vertical interrupted lines have the same significance as those in Fig. 8, *q.v.*

as possible from the kidney before resuming the normal return of venous blood to the caffeine-free reservoir. The kidney from the experiment represented in Fig. 7 was fibrosed.

6. *Sensitivity of caffeine diuresis to perfusion pressure.* Certain experiments in our series indicate that the diuretic action of caffeine is increased at higher perfusion pressure. Fig. 6, for example, shows a response of blood flow, but no diuresis at 108 mm. perfusion pressure, and

subsequently a well-marked diuretic response at 160 mm. Again, Fig. 4 shows that when perfusion pressures are kept adjusted to secure equal rates of urine secretion by the two kidneys, one of which is under the influence of caffeine, the difference of pressure is smaller at low than at high levels of perfusion pressure. Though this class of experiment has not been repeated often enough to enable the conclusion to be asserted with absolute confidence, it seems likely, and we have no evidence to the contrary, that the diuretic action of caffeine is enhanced by raising the perfusion pressure.

Discussion.

The salient conclusions to which our experimental results lead are, first, that the changes in the composition of urine secreted, under the experimental conditions described, are the same in kind and magnitude when produced either by caffeine or by increase of perfusion pressure, and, second, that an increase of blood flow, except when the dosage of caffeine is very small, is an integral part of the action of caffeine on the isolated kidney, and may be prominent even under conditions in which little or no diuretic response is obtained. These results could be simply explained by supposing that caffeine induced dilation of the vas afferens and efferens, but in suitable circumstances the effect on the former was the greater, and consequently the glomerular pressure was increased. It would be fruitless to discuss the mechanical aspects of such a system until the influence of pulse pressure had been studied. If caffeine increased the number of active renal units in the isolated kidney, as observed by Richards in the frog's kidney, it would be expected to yield more urine of substantially unaltered composition, which would be in conflict with the changes found in our experiments. If, on the other hand, caffeine acted by reducing the effective osmotic pressure of plasma proteins, it should exert more rather than less influence at low perfusion pressures, which is the reverse of the conclusion that seemed likely from our experiments. Moreover, the actions of caffeine in the concentrations described in this paper are, as has been seen, only partially reversible. None of the effects of caffeine recorded above necessitate a stimulation of a "vital" secretory process for their explanation, and a purely vascular hypothesis would seem adequate to account for them.

Summary.

1. The actions of caffeine have been studied on the single heart-lung-kidney and on the double heart-lung-kidney preparations of the dog.

2. Caffeine induces (i) an increase of perfusion flow at constant pressure, (ii) diuresis, (iii) an increase in chloride concentration of the urine, (iv) an increase in the amount of urea secreted in a given time, and decrease in its concentration.

3. When, in a pair of kidneys, a rise of perfusion pressure is arranged to induce diuresis in one, equal in amount to that induced by caffeine in the other, the compositions of the two urines are the same, whereas the blood flow through the organ affected by caffeine is greater.

4. The graded responses of the rates of urine flow and blood flow, induced by varying concentrations of caffeine, are illustrated.

5. The effects of a large dosage of caffeine are partly reversible, under the conditions described.

6. In certain experiments caffeine appeared to be more efficient as a diuretic at higher than at lower levels of perfusion pressure.

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THE SELECTIVE VASO-CONSTRICTOR ACTION OF ADRENALINE.

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The rise in blood-pressure following the injection of adrenaline into an animal is due to two factors: (1) increased cardiac output, and (2) constriction of vessels innervated by sympathetic vaso-constrictors. It is well known that the rise in pressure so produced calls into play the normal depressor reflexes so as to relieve the heart and arteries of excessive strain, and the heart may be slowed to such a degree that the rise in general blood-pressure is very small compared with that seen when adrenaline is given after section of the vagi. Apart from the cardiac slowing, however, reflex dilatation of vessels in the splanchnic area plays a considerable part in reducing the abnormally high pressure, for "the splanchnic nerve is the great vasomotor nerve to the body, through which the central nervous system regulates the general blood-pressure" (1). In 1895, as a result of their investigation of the action of adrenaline, Oliver and Schafer (2) stated "it may fairly be assumed that, although we are unable to record any plethysmographic observations upon the intestines, the great rise in blood-pressure which invariably follows the injection of the extract (of suprarenal gland) is in all cases due very largely to contraction of the arterioles in the splanchnic area," and ever since that time adrenaline has been credited with having a more powerful constrictor action on the intestinal vessels than elsewhere in the body; indeed, this conception forms an essential part of Cannon's view on the redistribution of blood in certain emotional disturbances (3). Observations of Pick (4), Loewi and Meyer (5), and Hartman (6) support the generally accepted view, but Schmid denies such predilection on the part of adrenaline (7). Taking the generally accepted view, however, there appears the picture, after an injection of adrenaline, of the normal reflexes of the body attempting to lower the blood-pressure by antagonizing vaso-constriction in that part of the body where it is most powerfully

produced. As the constriction is due to peripheral stimulation of vaso-constrictors no amount of central inhibition of vaso-constrictor tone is likely to have any effect, and the sole means of combating the condition, so far as present knowledge goes, is by stimulation of vaso-dilators. But it is generally recognized that the total action of dilator nerves is less than the total action of constrictor nerves in the splanchnic area (8), so that the picture portrayed above seemed unsatisfactory from an economic point of view, more especially as adrenaline is a natural product of the organism. In fact, in view of the necessity of keeping the vessels of the splanchnic area free to be acted on immediately and to any degree by reflexes controlling blood-pressure, it is conceivable that adrenaline would have less effect here than on other vessels.

Apart from the effects of very small doses under ether anæsthesia, there are records in the literature indicating that in the normal response to adrenaline the vessels in the intestine do not remain constricted throughout the whole of the period for which the blood-pressure is raised, but that during the later part of the period *the volume of the intestine is increased* (9, 10). This increase is sometimes not even mentioned when graphic records show it quite clearly (11). Hartman and Frazer (10) came to the conclusion that when dilatation occurred after a pressor dose of adrenaline it was due to an active nervous process, because they were unable to reproduce it after section of the splanchnic nerves or extirpation of the cœliac or superior mesenteric ganglia.

It is the purpose of the present paper to examine afresh the question of the selective activity of adrenaline on the splanchnic vessels. Cats were used, anæsthetized with chloralose, amytal or urethane, and in all cases at least 2 hours had elapsed between the administration of ether and the making of any experimental observations. Blood-pressure was recorded from the carotid artery by a mercury manometer, and volume changes in limbs and intestinal loops by means of plethysmographs connected to volume recorders of the type described by McDowall (12). Injections of adrenaline were made into the femoral vein, except when, with one limb in a plethysmograph, the cannula of a Bayliss compensator was inserted into the iliac artery of the opposite side; in these cases the external jugular vein was used.

The injection of adrenaline in sufficient amount to give a rise in blood-pressure always produced a fall of intestinal volume of short duration, succeeded by a more prolonged rise with a relatively slow return to the original volume (Fig. 1); when novirudin had previously been given to prevent clotting there was generally some permanent increase in volume,

and subsequently it was evident that this was due to increased transudation of lymph, as the lymph vessels were clearly seen to be engorged

A *B*

Fig. 1. Upper record—intestine volume. Lower record—blood-pressure. 0·01 mg. adrenaline at ↑. *A*, normal. *B*, liver not in circulation.

with fluid in these cases. In some experiments a transient preliminary increase in volume occurred, presumably due to action of adrenaline on the heart before reaching the intestinal vessels (Fig. 4 *C*), or to relaxation of the intestinal walls in certain cases (Fig. 2).

The shape of the curve recording changes in intestinal volume suggests either that the vaso-constrictor action of adrenaline on the vessels of the gut is remarkably transient, and is followed by a dilatation similar in nature to that seen after very small doses of adrenaline under ether anæsthesia, or that a more prolonged constrictor action is masked by some other factor which produces the increase in volume. It seemed more profitable to investigate the latter alternative, and to determine what conditions following an injection of adrenaline might cause increased intestinal volume. As the intestinal loop was tied at both ends, there can be no question of the greater volume being due to alteration in the contents of the gut unless the latter contained gas under pressure. In one or two experiments where the gut obviously contained gas, there was a rapid increase in volume after the injection of adrenaline, then followed a dip in the curve succeeded by the rise which is the subject of investigation; that is to say, the usual sequence of events took place, but at

a higher level (Fig. 2), and this was interpreted as indicating that relaxation of the intestinal muscle was responsible for the initial rise, but

Fig. 2. Cat; amytal anæsthesia. Vagi and splanchnics cut. Upper record—limb volume below knee. Middle record—blood-pressure. Lower record—intestine volume.

did not otherwise interfere with the phenomena. It seems, then, that the volume changes must be vascular in origin, but if this is so, during some part of the action of adrenaline there must be more blood in the intestinal area than normally. In order to investigate the problem, a review of the possible vascular causes of increase in intestinal volume suggested that one or more of the following might be responsible: (1) passive dilatation of veins and capillaries due to back pressure from the portal system; (2) dilatation of arteries or arterioles; (3) passive capillary or venous dilatation resulting from relaxation of the intestinal muscle. Each of these possible factors was investigated experimentally:

(1) It is well known that adrenaline causes a rise in portal venous pressure which may outlast the increase in general blood-pressure (7, 13, 14). To determine the part played by this factor the central end of the splenic vein was connected to the central end of the left renal vein by cannulæ and rubber tube, so that, on clamping the portal vein, the blood from the mesenteric area flowed directly into the vena cava; the splenic and left renal arteries were tied and clotting was prevented by injecting

novirudin intravenously. In such a preparation it was readily demonstrated that the volume of a loop of intestine after an injection of adrenaline showed similar changes whether the portal blood flowed only through the liver or only through the short-circuit (Fig. 1). It was thus evident that back pressure from the portal vein played little part in the observed volume increase in the gut.

(2) Direct measurement of the venous outflow from a loop of intestine showed that the flow was increased during the increase in gut volume. The loop was isolated by ligatures so that back pressure by way of anastomotic channels from the portal system could not be a contributory factor to the increased flow. Blood was allowed to fall from a cannula in the vein directly on to an electrical drop recorder, clotting being prevented by novirudin; the blood was returned at intervals through a cannula into the jugular vein. It will be seen from Fig. 3, that during

Fig. 3. Intestine volume and blood-pressure. Lowest record—drops of blood from branch of mesenteric vein.

the time corresponding to the peak of the rise in blood-pressure the venous outflow is diminished, but that very soon afterwards an obvious increase occurs which returns to the original rate as the pressure falls. The fact that the outflow falls when the blood-pressure is at its highest indicates that the constriction of the intestinal vessels is sufficiently intense to prevent the high pressure forcing more blood through the vessels, and in fact is directly contributory to the high pressure; this constriction, however, lasts a very short time, and is rapidly overcome by the blood-pressure, aided perhaps by dilator impulses, although with vagi cut these latter cannot be initiated by the depressor fibres. In one experiment

where the vagi were not cut, it was observed that the increased venous outflow was evident immediately the blood-pressure began to fall; during the fall in this case there was an abrupt drop, obviously due to cardiac slowing, and this was reflected in a slowing of the outflow from the branch of the mesenteric vein. It will be seen in Fig. 3 that the beginning of the increased rate of flow coincides with the point at which the intestine volume begins to increase; the gut volume does not fall in this case with the final decrease in blood flow because of the action of novirudin in facilitating lymph production already mentioned above.

It is of interest to observe here that, in several experiments where comparisons have been made, adrenaline has never produced the intense blanching of the intestine seen after a dose of vaso-pressin which gave the same or indeed a less rise in general blood-pressure.

(3) The possibility seems very remote that the third suggestion, namely, that relaxation of the gut walls exerts traction on the vessels and so dilates them, plays any part in the increase in intestinal volume. If this does occur, then the gut volume should increase after an injection of adrenaline whether the blood-pressure rises or not, because widening of the vessels by traction on their walls would simulate an active dilatation.

By connecting a Bayliss mercury compensator(15) with the central end of one iliac artery, and determining the response to adrenaline when no rise in blood-pressure occurred, it was found that there was no increase in intestinal volume but only a decrease of longer duration than that seen when the compensator connection was clamped; this difference is seen in Fig. 4. It is to be noted in these records that the line indicating intestine volume is sloping, pointing to slow continuous increase, a phenomenon almost invariably seen when novirudin has been given, and due, as previously stated, to transudation of lymph.

Fig. 5 gives further evidence of the vascular origin of the observed intestinal dilatation; here it is seen that stimulation of the peripheral end of the right splanchnic nerve immediately following the injection of adrenaline (Fig. 5 *B*) reduced not only the degree of dilatation but also the portal venous pressure, indicating that the blood flow had been reduced through that part of the intestine supplied by this nerve.

So far, then, the experimental evidence shows that during the vascular response produced by adrenaline there is normally a definite increase in intestinal volume, that this increase is due to a greater volume of blood in the intestine and entirely overshadows adrenaline vaso-constriction in the gut except in the initial stage of the action of the drug. The greater

Fig. 4. Cat; amytal anæsthesia. Intestine volume and blood-pressure. 0·01 mg. adrenaline at ↑. *A*, normal. *B*, Bayliss compensator in circuit. *C*, both splanchnics cut. *D*, both splanchnics cut and compensator in circuit.

volume of blood in the intestine is mainly, if not entirely, due to increased flow to the gut.

A B

Fig. 5. Cat; amytal anæsthesia. Upper record—portal vein pressure. Middle record—blood-pressure. Lower record—intestine volume. *S*, stimulation peripheral end right splanchnic. 0·01 mg. adrenaline at ↑.

In the next stage of the investigation it was necessary to see if the increase in intestinal volume depended entirely, as the work of Hartman and Frazer suggests (10), on a nervous mechanism. The splanchnic nerves were therefore exposed from the lumbar region and looped with thread in order to be readily available; the response to adrenaline was taken before and after cutting these nerves and it was evident that the changes in gut volume were qualitatively unaffected by the nerve section (Figs. 4 and 6). It will be noticed, however, that the dip in the volume curves after cutting the splanchnics (Figs. 4 *C* and 6 *C*) is accentuated, and this may be due either to the absence of the vaso-dilator effect of the depressor reflex or to the fact that the vessels, being cut off from the vasomotor centre, have lost their normal tone, and are therefore in a position to constrict to a greater relative extent than before in response to adrenaline. There is no doubt that, under the conditions of these experiments, the depressor reflex can overshadow the constrictor action of adrenaline on

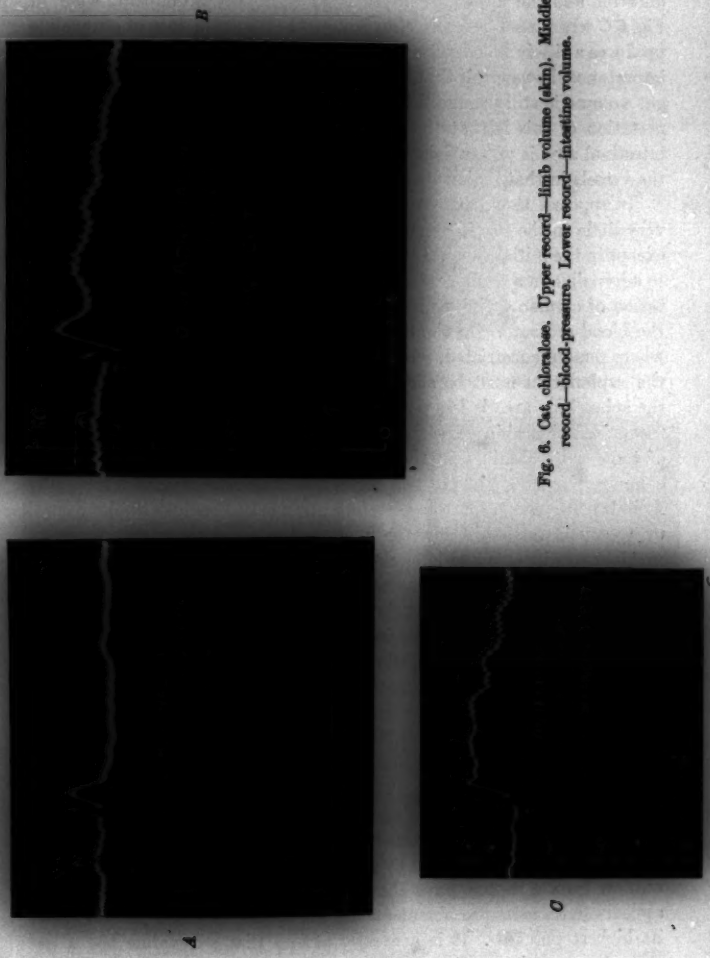

Fig. 6. Cat, chloralose. Upper record—limb volume (skin). Middle record—blood-pressure. Lower record—intestine volume.

the gut, because in Fig. 6 *A* the rise in blood-pressure is of very short duration and the intestine volume increase is very prominent, while in Fig. 6 *C* where no depressor reflex is possible the same dose of adrenaline produces a rise in blood-pressure of about 30 p.c. The point of immediate importance, however, is that, after section of the splanchnics, increase of gut volume is still produced by adrenaline, and the only possible interpretation of this is that the vaso-constrictor effect of the drug in the intestinal area is not sufficiently intense to prevent passive dilatation of the vessels in that region by the raised blood-pressure.

It appears, then, that vaso-constriction in the intestine contributes very little to the rise in general blood-pressure produced by adrenaline, except in the initial stage. If vessels in other regions of the body respond to adrenaline in a similar manner to those in the intestine, then augmentation of cardiac activity must be considered the main factor in raising the blood-pressure. On the other hand, if it is found that vessels elsewhere remain constricted, while the denervated intestinal vessels dilate, the explanation must be accepted that adrenaline vaso-constriction in the splanchnic area is less powerful than in other parts. It is generally recognized that skin vessels are constricted by adrenaline, but in view of the results obtained in the intestine it was considered necessary to compare the effects in the two regions of a pressor dose of adrenaline. A limb plethysmograph was therefore arranged to enclose the hind limb up to a point a little below the knee, so that as little muscle tissue as possible was included. Fig. 6 shows the changes in limb and intestinal volumes resulting from the same dose of adrenaline: *A*, with vagi and splanchnics intact; *B*, with vagi cut; and *C*, with both vagi and splanchnics cut; the volume changes in each case are qualitatively the same. There is no evidence of dilatation in the limb corresponding to that in the intestine; in the limb constriction dominates the picture, in the intestine dilatation. Similar volume changes in limb and intestine have been found to occur in the rabbit under chloralose anæsthesia (Fig. 7).

It has long been known that adrenaline may produce dilatation in a limb instead of constriction (2), and the explanation is accepted that this occurs only when there is a preponderance of muscular tissue inside the plethysmograph; if the skin has been previously removed from the limb, dilatation is always produced by adrenaline (16). Measurements of venous outflow from a muscle have shown an increase after adrenaline, which appears to be most prominent during the decline of blood-pressure from its highest point (17). It is generally agreed that adrenaline has a less powerful constrictor effect on the vessels of voluntary muscle than on

Fig. 7. Rabbit, chloralose. Vagi and depressor cut. Upper record—limb volume (skin). Middle record—blood-pressure. Lower record—intestine volume.

Fig. 8.

Fig. 9.

Fig. 8. Cat; decerebrated. Vagi and splanchnics cut. Upper record—volume of denervated limb (skin). Middle record—blood-pressure. Lower record—intestine volume. 0·01 mg. adrenaline at ↑, 2½ hours after end of ether administration.

Fig. 9. Cat; chloralose; vagi cut; Bayliss compensator in circuit. Upper record—volume of skinned limb. Middle record—blood-pressure. Lower record—intestine volume. 0·01 mg. adrenaline at ↑.

those of the splanchnic area (18), and experiments during the present investigation have confirmed this view in that only an increase in volume and no trace of a decrease, such as is seen in the intestines after the injection of adrenaline, could be observed in the skinned limb. By making use of the Bayliss mercury compensator, however, evidence was obtained that adrenaline has a very feeble vaso-constrictor action on the vessels of muscle, but that this effect gives way to dilatation, which seems to be active in type, in that it occurs when a rise of blood-pressure is prevented (Fig. 9); this dilatation, in many cases, lasted for 10 to 15 minutes or more. The long delay in the production of active dilatation in muscle under these conditions can readily be explained by recent observations of Hartman, Evans and Walker (19). By direct observation of the vessels in cat's muscle they found that intravenous injection of adrenaline (epinephrine) in amounts equal to or greater than 1 c.c. of 1 in 100,000 caused dilatation of capillaries but constriction of arteries, arterioles and veins. In the experiments with the Bayliss compensator this constriction of the larger vessels would mask the active dilatation of capillaries until the effect on the larger vessels began to diminish.

It appears, then, that of the three great vascular areas investigated, the skin is the only one that shows maintained vaso-constriction during the adrenaline rise in blood-pressure; contrary to the general belief there is actually more blood in the intestinal area during a considerable part of the adrenaline effect. It therefore follows that during muscular exercise, when a redistribution of blood occurs at the expense of the digestive system (3), adrenaline cannot be an important factor so far as its local action on the intestinal vessels is concerned, and nervous impulses passing directly to these vessels must play the greatest part. That splanchnic stimulation will reduce the vaso-dilatation during the adrenaline response has been shown in Fig. 5. The reactions described do not appear to be due to anæsthesia, for exactly similar changes are seen in the decerebrate cat 2½ hours after ether administration had ceased (Fig. 8); it has been observed, however, that adrenaline vaso-constriction in the intestinal region is less evident when amytal is the anæsthetic, and in some cases dilatation entirely obliterates the small amount of constriction produced (Fig. 5).

It is a little difficult to conceive that constriction of skin vessels alone, especially when this is accompanied by dilatation in intestines and muscles, can produce the rise in blood-pressure resulting from adrenaline action, and it must be conceded that increased cardiac output plays at

least an equal, if not greater part in the phenomenon. It is obvious, however, that this latter factor will be limited by the venous return to the heart, and unless this is increased to keep pace with the enhanced heart action the output will decrease. There is evidence that adrenaline constricts veins as well as other vessels (20, 21), and it may be that this constriction of veins plays no inconsiderable part in raising the blood-pressure by reducing the capacity of the venous system and facilitating the return of blood to the heart. Observations on man show that adrenaline usually raises venous pressure (22, 23), and in animal experiments a rise in pressure in the inferior vena cava has frequently been seen after adrenaline. Donegan (20) observed that the veins which gave the greatest response to adrenaline were those draining blood from the skin and those in the mesenteric region; muscle veins were unaffected and the inferior vena cava gave little or no response. Gunn and Chavasse (21), however, showed a definite contraction of the inferior vena cava with adrenaline.

If then the capacity of the venous system is reduced and the heart is stimulated to increased effort, further reduction of capacity of the vascular system by constriction of skin vessels appears to be sufficient cause for the rise in blood-pressure produced by adrenaline even though the vessels of muscle and gut are at the same time distended.

Summary and conclusions.

1. Adrenaline in amount great enough to raise the blood-pressure in cats and rabbits causes a prolonged vaso-constriction in skin vessels, but an increase in the volume of blood present in voluntary muscle and in the intestine.

2. The results in cats have been obtained on animals anæsthetized with chloralose, amytal and urethane and after decerebration.

3. The observed vaso-dilatation in the intestine is produced partly by reflex nervous means, but is also seen when all possibility of such reflexes is removed by section of vagi and splanchnic nerves; the dilatation can be diminished by electrical stimulation of the splanchnic. When a rise of blood-pressure is prevented adrenaline constricts the intestinal vessels, but the constriction is of a more transient and feebler nature than that seen in the skin. Back pressure from the portal system plays little part in the vaso-dilatation of the gut.

4. It seems that the rise in portal venous pressure following an injection of adrenaline must to some degree be enhanced by the increased blood flow through the splanchnic area.

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STUDIES ON DIFFUSION THROUGH SURVIVING ISOLATED INTESTINE.

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The process of absorption of soluble substances from the intestine has generally been investigated by determining either the amount of substance which disappears in a given time after placing the solution in an isolated or Thiry Vella loop in the living animal, or its concentration in the absorbing fluid. On account of the obvious difficulties, both experimental and chemical, standing in the way of successful application of the latter method, when the blood of the portal vein is used, we have sought for some other means by which the behaviour of absorbed substances might be studied, and the present paper embodies the results.

Diffusion is allowed to occur through the walls of isolated portions of intestine kept alive in a bath of oxygenated saline solution at body temperature. We shall show that under such conditions there is distinct evidence of selective absorption, and that this cannot be explained by known physico-chemical laws. Since there is no blood supply in the walls of the gut under these conditions and since, as Cori, Cori and Goltz(1) have shown, soluble substances are absorbed from the peritoneal cavity in obedience to physico-chemical laws, it seems permissible to assume that the selective property is a function of the mucous membrane. We were led to the use of this method through the observation, made by one of us (H. E. M.) in association with Southgate(2), that the movements of isolated pieces of intestine are but little affected by marked changes in the chemical composition or the H-ion concentration of solutions placed in the lumen, whereas, as is well known, they are extremely sensitive to changes in the outer fluid. These workers also showed that destruction of the vitality of the lining epithelium, by preliminary treatment with weak sodium fluoride solution, increased the permeability so that the movements became immediately susceptible to the influence of solutions placed in the lumen.

Methods.

Most of the experiments have been performed with the small intestine of about three-fourths full-grown rabbits. After a preliminary fasting period of not more than 15 hours, the animal was instantly killed by a blow on the head and a suitable length of intestine, for the number of observations to be made, excised and the contents removed by a gentle stream of water or, in the later experiments, normal saline at body temperature. Pieces, each about 15 cm. long, were then cut off and placed between filter paper moistened with Tyrode solution, their positions relative to the duodenum being carefully noted. Each portion was then weighed and, if necessary, trimmed so that all were of equal weight, after which they were tied at both ends on to short pieces of glass tubing passing through a cork, which was adjusted over a small beaker so that the loops were completely immersed in modified Tyrode solution—usually 50 c.c.—which was kept at body temperature and saturated with oxygen. In order to avoid damage to the gut wall, thick woollen yarn was used to tie the segments on to the tubes over which short pieces of rubber tubing had previously been drawn. The segments were tied carefully in two places. The bubbling of the oxygen kept the solution mixed. Equal volumes, usually 3–5 c.c., of the solutions under investigation were placed in each of the loops, care being taken to see that there was just enough to fill the loops without any rising into the glass tubing. The rate of diffusion was measured by analysis of samples (usually 2 c.c. each) of the outer fluid removed at suitable intervals. In calculating the number of milligrammes of diffused substance for each succeeding interval, allowance should be made for the previous removals of fluid, but in the present series of experiments this has not been done, since comparative results only were required. The methods used for analysis were as follows: for sugars, Shaffer-Hartmann; for calcium, Kramer and Tisdall; for phosphorus, Bell-Doisy; for iodine, Fellenberg-Leitch-Henderson; for hydroxyacetone, Campbell.

In observations lasting only a couple of hours, practically no protein appeared in the solution, at least not in sufficient amounts to interfere with the estimations by the above methods.

A factor which must obviously influence the rate of diffusion is the gradual decline in the strength of the solution in the loop and the rise in the outside fluid. In order to minimize this lessening of the diffusion-pressure gradient as much as possible, we have, as a rule, used in the

loops solutions that were as strong as possible and have given attention only to the diffusion occurring during a period of approximately 2 hours or so from the start. A better way to overcome this source of inaccuracy would have been to replace the diffusion fluid in the gut with fresh solution, but for the objects in view at the present stage of the research this was considered unnecessary. In a later paper we shall discuss results of experiments in which this procedure has been followed. The influence of microbial growth can be discounted in the majority of our experiments, since in 2 hours, which was their average duration, little destruction of material could have occurred. In observations of greater duration, in which glucose solutions were employed, a rapid disappearance of sugar became evident in the outer fluid, due, no doubt, to microbial destruction. In a few experiments which we have recently conducted with Purves, in which aseptic precautions were taken and the solution in the loop was constantly changed, no such disappearance of sugar has been observed in the outer fluid.

Even when solutions of the same strength were used it was noted early in the investigations that the diffusion rates might vary considerably through different portions of the same intestine, both for living and killed segments. An important cause for these differences has been found to be the level from which the piece of gut is taken, diffusion becoming more rapid, both for living and killed segments, in the aboral direction. This difference becomes very striking when the regions from which the segments are taken are far apart. Thus, in two segments, one taken from the duodenum and one from the ileum, each weighing 3·4 g. and containing 2 c.c. of a 0·2 p.c. solution of KI, the amounts of I, expressed as γI per c.c.[1] diffusing out at various intervals, were as follows:

		30 min.	60 min.	90 min.
Ileum	Living	5·2	8·2	11·8
	Killed	9·0	16·3	20·6
Duodenum	Living	0·4	2·4	2·8
	Killed	4·1	7·5	12·9

When adjacent portions of intestine are used and each is made as short as possible, the differences due to level are often not detectable, although in some cases, as the following results will serve to show, they may be quite apparent. A length of intestine was divided into seven portions of 3·6 g. each, and into the first, third and sixth, counting from the ileal end, were placed 2 c.c. of a 2*M* glucose solution. Diffusion was allowed

[1] γ equals 0·001 mg.

to proceed into 50 c.c. of glucose-free Tyrode solution at body temperature in each case with the following results:

	Mg. glucose per 100 c.c. of fluid.		
	30 min.	90 min.	120 min.
Segment nearest ileum	57	155	209
Third segment	50	142	187
Sixth segment	31	110	173

But even when allowance is made for the influence of level, and every other detail of the experiment is apparently exactly alike for each piece of intestine, there often still remains some considerable irregularity in the rates of diffusion through different pieces. To minimize this error as much as possible, our usual procedure has been to use alternating segments for the different conditions under investigation, and then to average the results obtained for each similar condition. Thus, when comparing the influence of temperature, six equal portions of intestine would be taken, the first, third and fifth being kept at one temperature, and the second, fourth and sixth at another. The average of the three results in each case was considered as representing the true result.

Results.

The results of a typical experiment comparing the diffusion of glucose through living and dead intestine are shown in curve form in Fig. 1.

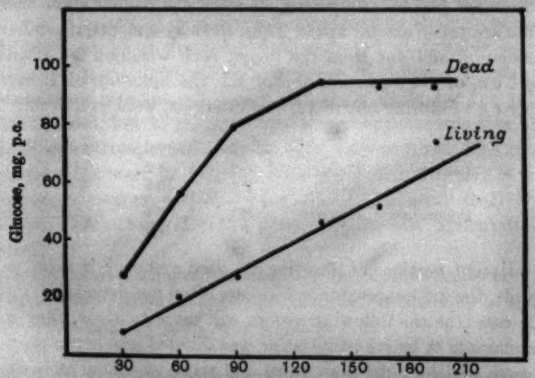

Fig. 1. 3 c.c. 4·5 p.c. in loops weighing 3·6 g.; 50 c.c. sugar-free Tyrode outside; 2 c.c. samples removed for analysis. The possible concentration in outside fluid when diffusion is complete is 135 mg. p.c. Abscissa, time in min.

3 c.c. of a 4·5 p.c. solution of glucose were placed in each of four loops, weighing 3·6 g., the first and third, counting from the ileal end, being killed by means of hot isotonic saline, and the second and fourth being alive. All loops were immersed in glucose-free Tyrode solution, 2 c.c. of which were removed for analysis at the intervals shown on the abscissa. The ordinates give the milligrammes of glucose present per 100 c.c. of outer fluid (the results for each pair of living and killed segments being averaged). The curves show that there was a striking difference in the rates of diffusion through the living and dead intestine. This was distinct after 30 minutes, but was most marked between the first and second hours, after which it became less owing, no doubt, to the fact that diffusion through the killed intestine became slowed down as the osmotic pressure rose in the fluid outside the loop. It will be noted that diffusion stopped in the killed loop when the concentration of glucose was about 100 mg. p.c., whereas according to calculation it should have been 135 mg. p.c. This difference, which we have always observed, is probably due to retention of sugar in the walls of the intestine. Because of this retention it is usually the case that no sugar can be detected in the outer fluid until some minutes after the preparation has been set up.

Through the living segment the rate of diffusion was practically constant during the 3 hours of the observation. Beyond this time, as a rule, the glucose concentration in the outer fluid began to fall rather rapidly, owing probably to organismal growth. As has already been said, we have not observed this falling off of glucose concentration when great care was taken to keep the outside solution sterile and the inside glucose solution constantly renewed.

On account of the complicating factor of bacterial destruction of glucose and other sugars, including di-hydroxyacetone, it is interesting to compare the diffusion of iodine through living and killed intestine, and the results of such experiments are shown in curve form in Fig. 2. It will be seen that up to $4\frac{1}{2}$ hours the diffusion proceeded in the same way as with glucose except that there was a slight falling off in the rate in the living segments. At the end of the experiment the outside fluid contained 31γ of iodine per c.c., whereas, at equilibrium, there should have been about 44γ after making allowance for the reduction in volume in the outer fluid and the removal of iodine in the samples. The most important fact that comes to light by comparison of the curves for glucose and KI is that they are very much alike, and this would seem to contradict the view that it is because of some physiological property of the surviving intestine that diffusion through it differs from that through the dead.

The results so far can be adequately explained as due to differences in the physical permeability of the walls, but it will be shown in the remaining part of this paper that another factor—selective absorption—can also be demonstrated for the living intestine.

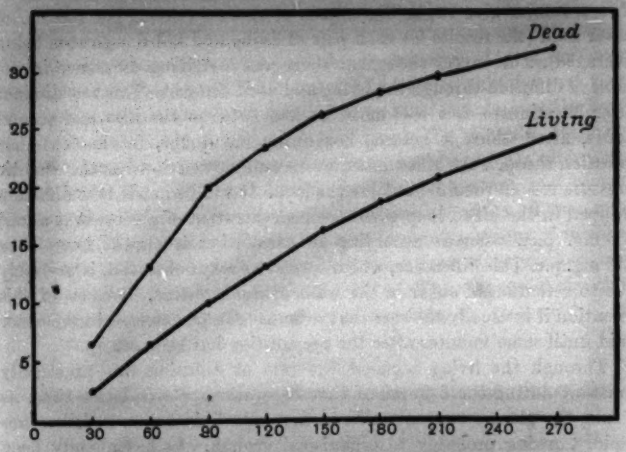

Fig. 2. Rate of diffusion of 2 c.c. 0·2 p.c. KI placed in loops weighing 4·3 g.; 100 c.c. Tyrode outside; 2 c.c. samples removed. The possible concentration at equilibrium would be 44γ. Ordinates, γI per c.c. Abscissa, minutes.

Influence of mode of killing on rate of diffusion.

The more rapid diffusion which occurs through the intestine after killing it by heat, than through living intestine, is no doubt due to a change in the physical texture of its walls. Heat, by coagulating the protein, may change the membrane from being of the nature of an emulsoid to that of a suspensoid. It became of interest to see whether similar changes in permeability occurred by using other means than heating to bring about death. This has been done in the following experiments, in which quantities of 3–4 c.c. each of a *M*/4 glucose solution were placed in a series of neighbouring segments of gut each having previously been treated in different ways, and diffusion was allowed to occur at body temperature into 50 c.c. glucose-free Tyrode solution.

The substances used for killing the intestine were:

(1) Saline at 65° C. (Placed in the segment for 4 minutes.)

(2) 2·5 p.c. NaF solution. (Placed in the segment for 4 minutes and then washed out with Tyrode solution.)

(3) 0·5 p.c. NaCN solution. (Placed in the segment for 2 minutes and then washed out six times with Tyrode solution.)

(4) Turning the segment inside out and exposing the mucosa to warm air for about 20 minutes. The segment was then reverted before tying on the tubes. The exposed mucosa never became dry, apparently owing to secretion of mucus.

The following table shows the results of three typical experiments, in which it is to be noted that each succeeding segment is farther removed from the ileum so that the permeability decreases from above down.

TABLE I. Mg. p.c. of sugar in outside fluid.

Exp. 1.	Time in min. ...	*20*	*40*	*60*	*80*	*100*	
	(1) Heat killed	50	82	82	109	109	Ileal end
	(2) Living	26	41	62	90	93	
	(3) NaCN	18	38	80	93	98	
	(4) Heat killed	47	80	101	(96)	109	
	(5) Living	15	24	36	(94)	66	
	(6) NaCN	19	43	80	90	88	Duodenal end

Exp. 2.	Time in min.	*30*	*45*	*60*	*90*
	(1) NaF	2	9	18	26
	(2) Living	3	8	14	15
	(3) Reversed and dried	21	32	38	42
Exp. 3.					
	(1) NaF	6	14	27	28
	(2) Living	5	6	14	18

Although heat caused the greatest increase in permeability, the other methods usually also had this effect. In Exp. 1 cyanide did not affect the rate of diffusion in one of the segments in which it was used, but it increased it almost to the same extent as heat in another. Exposure of the everted intestine greatly increased the permeability, but it proved to be very difficult to perform the eversion without mechanical injury to the intestine. NaF usually gave results similar to those shown in the table and seems to be the most dependable method next to heat.

Similar experiments were carried out, using dihydroxyacetone in place of glucose. Since NaCN combines with this substance, great care had to be taken to see that all was removed from the intestine, by washing before starting the diffusion. The results were much the same as with glucose in showing that fluoride and cyanide usually caused much the same increase in permeability as heat.

COMPARISON OF XYLOSE AND GLUCOSE.

1. *Rabbit intestine.* Equal volumes (3–4 c.c.) of equimolecular solutions of xylose or glucose were placed in pieces of intestine (jejunum) of approximately similar weights and the possible influence of level on the rate of diffusion was allowed for by using two pieces for each sugar arranged in the following order: beginning at the ileal end, glucose, xylose, glucose, xylose or *vice versa*. Each piece was immersed in 50 c.c. oxygenated sugar-free Tyrode solution kept at body temperature, and samples of 5 c.c. each of the outer fluid were removed at intervals for analysis. There was as little delay as possible in weighing and setting up the preparations. Results for dead segments are shown in Table II.

TABLE II. Intestine killed by heat (4 c.c. *M*/2 solutions placed in loops).

Time in min.		*30*	*60*	*90*	*120*
Mg. p.c. GLUCOSE in outer fluid	Loop 1*	15	34	56	71
	Loop 3	20	41	66	82
	Av.	17·5	37·5	61	76
Mg. p.c. XYLOSE in outer fluid	Loop 2	31	68	120	137
	Loop 4	52	93	164	167
	Av.	42	81	141	152

* The loops are numbered from the ileal end.

In this case, contrary to the usual experience, both sugars diffused somewhat more rapidly as the loops were farther removed from the ileum and the average rate, as judged by the increase of percentage in the outer fluid, was about twice as great for xylose as for glucose.

In another experiment of the same nature the average number of milligrams p.c. of sugar in the outer fluids at half hourly intervals was as follows:

Glucose	7	16	29	36
Xylose	16	34	47	66

A typical result for living pieces of intestine in otherwise exactly similar experiments is shown in curve form in Fig. 3. Sometimes in the case of rabbit intestine diffusion of the two sugars was nearly equal, as is shown in Table III.

TABLE III. 4 c.c. *M*/2 solution placed in loops.

Time in min.		*30*	*60*	*90*	*120*
Mg. p.c. GLUCOSE in outer fluid	Loop 1	27	43	65	101
	Loop 3	22	43	65	102
	Av.	24·5	43	65	101
Mg. p.c. XYLOSE in outer fluid	Loop 2	28	47	—	115
	Loop 4	20	41	64	95
	Av.	24	44	64	105

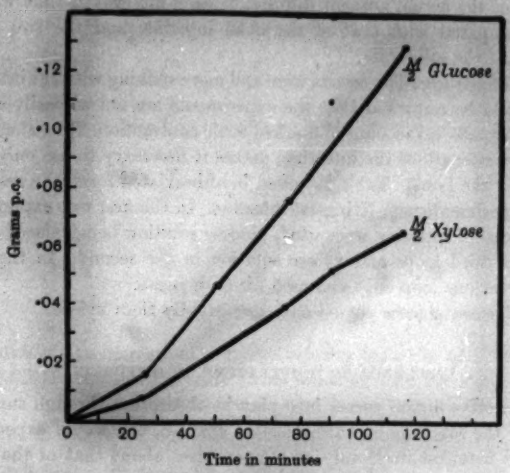

Fig. 3. Rates of diffusion of *M*/2 solutions of glucose and xylose through living intestine of the rabbit.

TABLE IV.

Time in min.		*30*	*60*	*90*	*135*	*190*	
(1) Mg. p.c. GLUCOSE in outer fluid	Loop 1	—	14	35	82	104	Young kitten. 1 c.c. 50 p.c. solution of sugar used in each loop
	Loop 3	—	13	26	57	86	
	Av.	—	14	30	69	95	
Mg. p.c. XYLOSE in outer fluid	Loop 2	—	9	18	44	78	
(2) Mg. p.c. GLUCOSE in outer fluid	Loop 1	29	71	103	—	—	do. do.
	Loop 3	—	43	72	—	—	
	Av.	29	57	87	—	—	
Mg. p.c. XYLOSE in outer fluid	Loop 2	14	33	59	—	—	
(3) Mg. p.c. GLUCOSE	Loop 1	7	14	40	—	—	Young kitten. 1 c.c. *M*/1 sugar solution used in each loop
Mg. p.c. XYLOSE in outer fluid	Loop 2	3	10	18	—	—	

There was very little difference in diffusion according to the level of the loops; the actual amount diffused at each interval of time was less when compared with that of the dead intestine and the two sugars diffused at the same rate.

2. *Cat intestine.* The results were still more striking with cat intestine, but it is to be remarked that the experiments are not so easily carried out in this case, on account of marked tonic contraction. This, along with the shorter length of the intestine, makes it necessary to use only 1 c.c. of fluid in the lumen. Table IV gives, in abbreviated form, the results of three experiments with living cat intestine. In the first two experiments three loops of intestine were used, glucose solution being placed in the first and third loops and xylose solution in the second. In the third experiment one loop only was used for each sugar.

In all cases glucose passed out more rapidly than xylose.

Influence of temperature on diffusion.

If selective action comes into play in absorption through surviving loops of the intestine of warm-blooded animals, one would expect that it would manifest itself only at temperatures about that of the body, whereas, at very low temperatures, physical factors would alone come into play. As the temperature was raised, increase in the rate of diffusion would not be the same when the intestine was alive as when it was dead. We have used two types of experiment in this connection, as follows:

(1) Comparison of the rates of diffusion of glucose solutions at 0°, 20° and 40° C.

(2) Comparison of the rates of diffusion of equimolecular solutions of glucose and xylose at 0°, 20° and 40° C., through loops of living and dead intestine.

Comparison of rates of diffusion of glucose solutions at different temperatures.

In these experiments, six segments of equal weights were taken, each containing 3 c.c. of glucose solution and immersed in 50 c.c. of sugar-free Tyrode solution which was kept at a temperature of 40° C. in the case of the 2nd, 4th and 6th segments and at about 0° C. in the 1st, 3rd and 5th segments. By averaging the results for each group of three segments at the different temperatures, the influence of level is discounted and these averages are given in Table V. The observations were made with living and dead intestine.

TABLE V. Mg. glucose p.c. diffusing at different temperatures.

	Strength glucose	Temp.	30 min.	60 min.	90 min.	120 min.
				Segments living		
(1)	3*M*/4	0°	7	25	—	62
		40°	30	67	—	172
(2)	3*M*/4	0°	6	20	40	—
		40°	30	64	105	—
(3)	2*M*	0°*	14	—	56	85
		40°	46	—	136	189
(4)	*M*/4	0°	—	15	31	47
		40°	—	38	68	119
				Segments killed		
(5)	3*M*/4	0°	8	31	—	66
		40°	25	64	—	136
(6)	3*M*/4	0°	6*	62	91	—
		40°	—	107	279	—

* Only two results averaged.

The ratios of the increase in diffusion velocity for a rise of 40° C. in the case of 3*M*/4 solutions placed in living intestine were therefore:

For living intestine after	30	minutes		4·5 and 5
do.	do.	60	do.	2·7 and 3·2
do.	do.	90	do.	2·6
do.	do.	120	do.	2·8
For dead intestine after	30	do.		3·2 (and 13·5)
do.	do.	60	do.	2·0 and 1·7
do.	do.	90	do.	3·0
do.	do.	120	do.	2·1

The corresponding ratios for simple diffusion of an inorganic salt (NaCl) would be about 1·57 and for a chemical reaction about 16 (van't Hoff)[1]. In the majority of cases the ratio is somewhat greater for living than for dead intestine, the only exception being after 90 minutes diffusion.

In the following experiments (Table VI), the rates of diffusion were

TABLE VI. Mg. glucose p.c. diffusing at different temperatures.

Exp. A.	30 min.		60 min.		90 min.	
Temp.	L.	D.	L.	D.	L.	D.
0°	11·25	12	26	41·5	44	86·5
20°	22·7	34·3	46	91	70	154
40°	87·2	63	163	171	209	204·5
Exp. B.						
0°	2·25	7·25	12	21	52	74·7
20°	6·7	16·5	46	37·5	86·5	112·5
40°	66	22·7	142	67·7	207	169·5

[1] Lewis, W. C. Mc., Physical Chemistry (1921), vol. 1, p. 381. Longmans, Green & Co.

measured at 0°, 20° and 40° C. for both living and dead segments, 4 c.c. 3*M*/4 glucose solution being placed in each loop. The dead segments were nearer the duodenal end.

As in the previous experiments the ratios of increase are higher for living than for dead intestine, as will be clear from Table VII, in which the amounts diffusing at 0° C. are taken as unity.

TABLE VII. Ratios of diffusion taking the amount at 0° C. as unity.

Exp. A.	30 min.		60 min.		90 min.	
Temp.	L.	D.	L.	D.	L.	D.
20°	2·03	2·86	1·95	2·19	1·6	1·78
40°	7·8	5·24	7·03	4·12	4·8	2·36
Exp. B.						
20°	3·0	2·2	3·83	1·9	1·66	1·5
40°	29·3	3·1	11·8	3·2	4·0	2·3

The ratios of increase between 0° and 20° C., with one exception, are much the same for living and dead intestine, but those between 0° and 40° C. are decidedly greater for the former. This difference occurred at each interval of observation, and it would seem to indicate that the epithelium allows glucose to diffuse more rapidly as its vitality is stimulated by rise of temperature. It will be observed that the values of the ratios for the range between 0° and 20° C. are not very far removed from those for a physical reaction (1·56) for both living and dead segments, but that those for between 0° and 40° C. are decidedly above the theoretical value of 2·44 in the case of living segments. We will discuss the significance of this difference after we have considered the other results.

Comparison of effects of temperature on diffusion of xylose and glucose.

We have seen that glucose diffuses more rapidly than xylose when equimolecular solutions of the two sugars are placed in surviving intestine kept at 40° C., but that xylose diffuses more rapidly than glucose through dead intestine. It became of interest, therefore, to compare the relative rates of diffusion of these two sugars through segments of the same intestine at 40° C. and 0° C. At the lower temperature it would be expected that any "vital" properties of the mucosa would disappear and the relative rates of diffusion become similar to those of killed intestine.

The following experiment (Table VIII), in which 50 p.c. solutions of both sugars were used, demonstrates this. Four pieces of intestine of the cat, each weighing 12·2 g., were arranged in the usual manner and 1 c.c.

of 50 p.c. solutions of either glucose or xylose placed in them. The outer baths were kept at either 40° C. or 0° C.

TABLE VIII. Mg. sugar per 100 c.c. in outer fluid.

	60 min.	120 min.	180 min.	240 min.
Glucose 0° C.	0	7	20	45
Xylose 0° C.	2	7	21	37
Glucose 40° C.	10	32	66	145
Xylose 40° C.	5	18	41	81

It can be seen that whereas diffusion occurred at practically the same rate for the two sugars at 0° C., glucose diffused more rapidly than xylose at 40° C. Since we have already given a sufficient number of results to show that glucose diffuses more rapidly than xylose at 40° C., it will be necessary to add here only some further observations (Table IX) with xylose and glucose at 0° C.

TABLE IX. Mg. sugar per 100 c.c. of outer fluid at 0° C.

Animal used	Strength of solution in intestine	Sugar	60 min.	90 min.	120 min.	180 min.
Rabbit	3*M*/4	Glucose	22	34	—	—
		Xylose	11	20	—	—
„	3*M*/4	Glucose	16	32	—	—
		Xylose	6	18	—	—
Cat	3*M*/4	Glucose	—	—	11	32
		Xylose	—	—	10	23
„	3*M*/4	Glucose	10	—	13	19
		Xylose	8	—	9	18
„	50 p.c.	Glucose	2	—	16	19
		Xylose	1	4	16	32
„	3*M*/4	Glucose	1	—	4	13
		Xylose	2	—	13	31
„	3*M*	Glucose	1	—	4	12
		Xylose	1	—	6	19
„	3*M*	Glucose	1	—	1·5	7
		Xylose	1	—	4	16

The observations in which the rabbit intestine was used showed that on the whole, although glucose diffused more rapidly than xylose, it did not do so to the same degree as at higher temperatures. With the cat intestine at the low temperature both sugars diffused either at the same rate or xylose diffused more rapidly.

INFLUENCE OF STRENGTH OF SOLUTION ON DIFFUSION.

Because of the varying permeability of its different levels it is impossible, either in living or in dead intestine, to determine the exact relationship between concentration of the dissolved substance and the

rate of diffusion. An approximate comparison must nevertheless be of interest, since one would expect the rate of diffusion through the dead membrane to run parallel with change in concentration, whereas, in the living, it would either be optimal within some more or less restricted range of concentration, or be independent of this within wide limits. In carrying out experiments bearing on this part of our problem, it will have to be remembered that the surviving gut is only partly living, so even when selective absorption is at its highest physical diffusion through its walls will always be occurring.

The experiments with glucose may be divided into two groups according to the range of strength of solutions used. In the first group of experiments the strengths of the glucose solutions were $M/1$, $3M/4$ and $M/2$. The outstanding result, obtained in all of them, was that diffusion through the living gut was less rapid with a $M/1$ than with a $3M/4$ solution, whereas it was greater through the dead.

The results of a typical experiment are shown in Table X and also in curves (Fig. 4).

TABLE X. Mg. p.c. glucose in outer fluid*.

Exp. No.	Time after start ...	Living 30 min.	Living 60 min.	Living 90 min.	Dead 30 min.	Dead 60 min.	Dead 90 min.
(1)	$M/2$ glucose	17	43	78	22	58	118
(2)	$3M/4$ glucose	49	133	252	41	89	178
Difference between 2 and 1		32	90	174	19	31	60
(3)	$M/1$ glucose	29	95	202	64	132	270
Difference between 3 and 2		−20	−38	−50	23	43	92

* Minus sign indicates that less sugar diffused from the stronger solution.

Diffusion of glucose through the living gut under the conditions of these experiments was therefore optimal at or about a concentration of $3M/4$, and at higher concentrations there was often less diffusion, at least up to solutions of the strength of $1M$. We have obtained similar results in about one-half of the experiments of this type which we have performed. Thus, in eight out of a total of sixteen experiments, in which all sugar analyses were done in duplicate, diffusion from a $1M$ solution was either less rapid than, or the same as from a $3M/4$ solution. Several of these experiments were independently conducted by Reid, an example of whose results is added, since it shows the further fact that the phenomenon may be more marked in segments taken near the duodenum than in those near the ileum. In order to make this clear the table is constructed on a somewhat different plan so as to indicate the relative positions of the segments (Table XI).

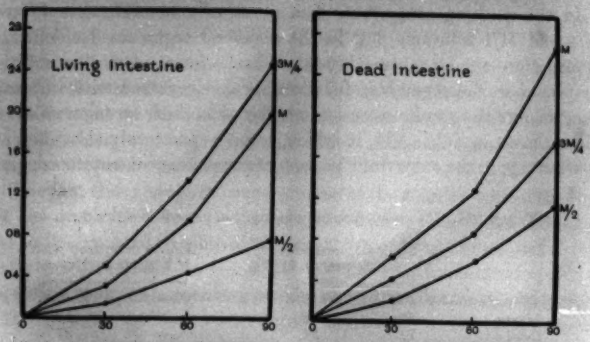

Fig. 4. Diffusion of glucose solutions of varying strengths through living and dead intestine. Ordinates, mg. p.c. glucose. Abscissa, time in min.

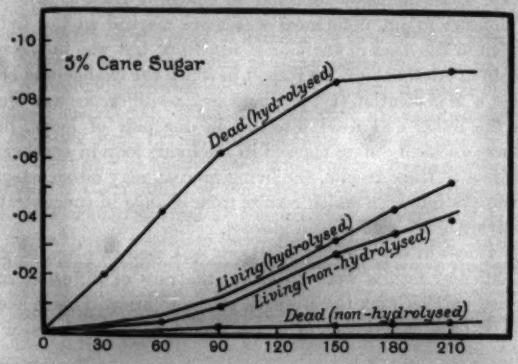

Fig. 5. Rate of diffusion of 5 c.c. 5 p.c. cane sugar solution through portions of living and killed intestine. Ordinates, mg. p.c. glucose. Abscissa, time in min.

TABLE XI. Mg. p.c. glucose diffusing from glucose solutions of varying strengths placed in living segments arranged as follows.

Ileum	1	2	3		4	5	6	Duodenum
	M/2	3*M*/4	*M*/1		*M*/2	3*M*/4	*M*/1	
30 min.	67	79	82		43	90	76	
60 „	130	178	170		86	178	131	
90 „	165	190	192		110	184	164	
150 „	344	372	360		198	350	295	

In the ileal segments there was practically no difference between $3M/4$ and $M/1$ solutions, but in the duodenal segments decidedly less diffused from the stronger solution. When a series of weaker solutions was placed in the segments, diffusion always increased with increasing strength, but the results were less regular than with stronger solutions. This is shown in Table XII, in which, to save space, only the differences in percentage in the outer fluid for each change of concentration are given.

TABLE XII. Difference (increase) in mg. p.c. glucose in outer fluid.

	$M/2$ as compared with $M/3$ solution (Exp. 4)		$M/3$ as compared with $M/4$ solution (Exp. 5)	
Time after start ...	60 min.	90 min.	60 min.	90 min.
Living	33	46	—	29
Dead	13	24	—	27
Living	8	2	17	28
Dead	17	15	10	18

In Exp. 4 the increased diffusion on changing from a $M/3$ to $M/2$ glucose solution (3 p.c. difference) was more marked in the living intestine than in the dead in one pair of segments, but was in the reverse relationship in another pair. In Exp. 5, in which the change was between a $M/4$ and a $M/3$ solution (1·5 p.c. increase), no difference was observed between the living and dead intestine in one pair of loops, but the increase was decidedly more marked in the living loop in another pair.

According to these results, the living mucosa may either exaggerate or suppress the influence which results from change in concentration of comparatively dilute solutions of glucose.

When xylose in similar concentrations was used in place of glucose entirely different results were obtained, the rate of diffusion running more or less parallel with the strength of the solution. This is shown in Table XIII, each experiment of which was done with pieces of living

TABLE XIII. Difference (increase) in mg. p.c. xylose in outer fluid. (Intestine living.)

	$3M/4$ as compared with $M/2$ solution			$M/1$ as compared with $3M/4$ solution		
Exp. No.	30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
1	—	—	—	16	24	—
2	7	15	17	6	12	15
3	10	20	40	22	16	38
	Between $M/4$ and $M/3$			Between $M/3$ and $M/2$		
4	0	1	3	13	14	15
5	7	21	30	1	9	22

intestine taken from the same animals as those used for glucose, as indicated by the numbers in Tables X and XII.

Although there is considerable irregularity in the figures, especially after 30 minutes' diffusion, it is nevertheless clear that the rate increases as the strength of the xylose solution becomes greater and that there is no optimal concentration beyond which diffusion remains steady or becomes less, as is the case with glucose.

The fact that change in concentration of the solution within the living gut has much less effect on the rate of diffusion than through dead gut was to be expected in view of the results obtained by Cori and Cori(8). These workers found in the white rat that the amount of sugar disappearing from the alimentary canal was, within wide limits, independent of the strength of solution introduced into it and was the same from hour to hour, although, of course, the solution remaining unabsorbed was becoming weaker. If similar results had been obtained in our experiments, there should have been no increase in rate of diffusion as the strength of the sugar solution was increased, whereas, as the tables show, this occurred for all the steps of increase up to a $3M/4$ solution, beyond which, however, in the majority of experiments with glucose, diffusion, instead of becoming greater, actually became less. In other experiments it remained unchanged. When dead intestine was used with glucose, or living intestine with xylose, this optimum concentration for diffusion was not observed. The phenomenon is difficult to explain unless we assume that under the conditions of our experiments the selective absorption of glucose becomes optimal at concentrations around $3M/4$, which is much above that of a solution isotonic with blood plasma. At concentrations below this optimum, migration of glucose through the walls of the living gut should increase with increasing concentrations more rapidly than through dead gut, because selective absorption would be progressively adding its effect to that of increasing diffusion due to physical causes alone. We have not as yet demonstrated if this be so. At higher concentrations than $3M/4$, we must imagine that the power of selective absorption becomes depressed or inhibited, so that the movement outwards of glucose falls off, being dependent on physical diffusion alone. We have tried to throw further light on this problem by measuring the volume of the solutions remaining in the loops of intestine after the experiments, but the results, which are only approximate because of technical difficulties, have not been of assistance. It will be necessary to repeat these measurements of volume at more frequent intervals during the course of the experiments. Although this part of our work is

not yet completed, the results so far obtained are sufficiently definite to be given as evidence that some property of selective absorption is manifested by the walls of the surviving intestine.

Finally, we will add some results obtained when cane sugar and substances other than carbohydrates are used.

Cane sugar. As would be expected, not only does cane sugar diffuse more rapidly through dead than through living intestine but it becomes inverted in the latter case without undergoing any significant change in the former. This difference depends, of course, on the presence of invertase, and it is of interest that it was not observed when sodium fluoride was used as the killing agent. The results of a typical experiment are shown in curve form in Fig. 5, and were obtained by allowing 5 c.c. of 5 p.c. cane sugar solution to diffuse into 100 c.c. Tyrode solution, of which 2 c.c. samples were then removed at intervals and the reducing power determined, either directly (unhydrolysed) or after heating with two drops of HCl (conc.) for half an hour and then neutralizing. The results point the way to a new method by which the action of intestinal ferments may be investigated. Work in this direction is now in progress.

Diffusion of other substances than the sugars.

The diffusion of various other substances, usually dissolved in saline solution isotonic with the Tyrode, has also been investigated. These may be arranged as follows:

(1) Acids, including acid phosphates.
(2) Potassium iodide.
(3) Dihydroxyacetone.
(4) Dye stuffs.

In a general way the results obtained with all of these substances, except the dye stuffs, showed more rapid diffusion through killed than through living intestine, the dye stuffs failing to diffuse in either case. This suggests that the porosity of the membrane is the factor alone concerned in determining diffusion and that it becomes increased by killing, by whatever means this is performed, since the same results are obtained whether heat, sodium fluoride or exposure of the mucosa to air is used as the killing agent. Certain of the results with phosphate suggest, however, that other factors also enter in to determine diffusion of the phosphate ion. On account of certain experimental difficulties, we have not as yet succeeded in obtaining satisfactory results when inorganic salts, such as those of calcium and magnesium, are used. Further in-

vestigation in this direction remains for the future. Typical results with the substances listed above may now be given in brief.

Acids. The difference between living and dead intestine can be strikingly demonstrated by observing the change in tint produced when a few drops of B.D.H. universal indicator are placed in the Tyrode solution in which the loop of intestine containing acid is suspended. In the case of the killed loop, acid begins to penetrate outwards almost immediately, as revealed by a reddish tint appearing along the edges of the loop, whereas no visible change occurs for some time when the loop is alive. After the change of tint has spread throughout the Tyrode solution, it can be brought back to the original green by the addition of alkali and the observation repeated.

The rate of penetration of acid can be conveniently followed in samples of the Tyrode solution either by colorimetric measurement of the *p*H or by titration with weak alkali, the usual buffer salts being omitted from the Tyrode solution. The most satisfactory results were obtained when 5–6 c.c. 0·5 p.c. solution of NaH_2PO_4 (*p*H 2·4) were placed in the lumen of a piece of gut weighing about 5 g., and 100 c.c. of buffer-free Tyrode were placed outside. The following are typical results:

c.c. *N*/100 NaOH required to develop pink tint in 2 c.c. of outer fluid containing phenolphthalein.

	10 min.	30 min.	40 min.	80 min.	100 min.	120 min.
Living loop	0·09	0·13	0·17	0·23	0·30	0·56
Killed loop	0·16	0·24	0·26	0·35	0·46	0·62

It will be noted that the difference between the living and dead loops became suddenly less after about 100 minutes, due no doubt to the fact that the living gut wall had died.

The fact that any penetration of acid occurs through living intestine is probably owing to the fact that it is partly dead, and it is of interest that much more complete delay in the diffusion of acid can be observed (by the universal indicator method) when the excised stomach and intestines of the frog are used. It is possible also that the presence of mucus in the living gut is partly responsible for the delay in diffusion of acid.

When solutions of phosphate (NaH_2PO_4) are placed in the loops and the outward diffusion of phosphorus is observed, the results are irregular. Thus out of ten experiments in which equal amounts of 0·5 p.c. NaH_2PO_4 were placed in living and dead (adjacent) loops immersed in phosphate-free Tyrode solution, there was no difference between living and dead in three, diffusion was more rapid through the living in four and more rapid

through the dead in three. Even when diffusion of this ion was compared through two neighbouring loops of killed intestine, uniform results were not obtained, and we have been unable to discover the cause for the irregularity.

Potassium iodide. The differences between living and dead intestine are here very marked, as has been illustrated in a table of results previously published. Temperature was also found to affect the comparative rates of diffusion of living and dead segments, as is illustrated in the following table, in which are given the number of c.c. of $N/100$ thiosulphate required to decolorize the iodine in 2 c.c. quantities of the outside fluid removed at the intervals shown, when 5 c.c. 0·2 p.c. KI solution were placed in each of the loops.

		30 min.	65 min.	90 min.	115 min.	145 min.	
Bath at 40° C.	L.	0·20	0·49	0·61	0·75	0·87	This shows that retardation of diffusion occurs at body temperature in the living gut
	D.	0·21	0·58	0·81	0·99	1·2	
Bath at 0° C.	L.	0·11	0·36	0·50	0·56	0·75	
	D.	0·11	0·31	0·48	0·61	0·75	

With this salt the rate of diffusion was found to be proportional to the strength of solution placed in the intestine, there being no difference between living and dead loops in this regard. Thus, when 5 c.c. of a 0·1 p.c. KI solution were placed in one pair of loops and 5 c.c. of a 0·2 p.c. solution in another, the amounts of I expressed as γ (0·001 mg.) per 100 c.c. were as follows:

		30 min.	60 min.	90 min.	120 min.	280 min.
0·2 p.c. KI	L.	13·5	25·8	35·3	46·2	65·4
	D.	25·8	42·0	55·3	62·1	71·0
0·1 p.c. KI	L.	8·80	12·9	18·1	22·4	32·5
	D.	15·50	24·3	28·6	33·5	36·8

It can be seen that roughly twice as much iodine diffused out from the stronger solutions. So far as this salt is concerned, therefore, there is no evidence of any selective absorbability on the part of the living intestine.

Dihydroxyacetone. The importance of studying the diffusion of this triose rests on the fact that it has been observed by Cori and Cori(8) to be absorbed more slowly than glucose from the alimentary canal of the rat. This difference cannot, however, be demonstrated with surviving intestine. Allusion may here be made to observations in which the influence of temperature on the rate of diffusion was studied. Typical results are shown in the following table, in which the figures represent

the c.c. *N*/100 permanganate required to oxidize the reduced Folin Wu reagent in 1 c.c. samples of the outer fluid. The loops of intestine were all of the same weight, those at the higher temperatures being nearest the ileum.

		40 min.	80 min.	120 min.	160 min.
39° C.	L.	0·10	0·19	0·33	0·36
	D.	0·18	0·34	0·74	0·65
27° C.	L.	0·06	0·10	0·20	0·22
	D.	0·13	0·25	0·43	0·44
15° C.	L.	0·05	0·13	0·17	0·19
	D.	0·08	0·17	0·32	0·34
3° C.	L.	0	0·05	0·12	0·13
	D.	0·08	0·15	0·38	—

It can be seen that as the temperature was raised the rate of diffusion did not increase more rapidly in living than in dead intestine, a result which is contrary to those obtained with glucose (*vide supra*).

Dye stuffs. Both the dead and the living intestinal loops were entirely impervious to dyes. With acid fuchsin and methylene blue, the walls of the dead gut became stained more deeply than those of the living, but none of the tint spread to the outside fluid.

Discussion.

The more rapid diffusion which occurs through dead as compared with surviving intestinal loops is due to changes of a physico-chemical nature in the walls. Whatever means are adopted to kill the intestine (heat, NaF, KCN, exposure to air) the same increase in permeability is seen to occur towards all diffusible substances (sugars, KI, dihydroxyacetone, acids, etc.), and, no doubt, coagulation of protein with a consequent change in the colloidal structure of the intestinal walls is mainly responsible. It is possible that an increase in the area of the diffusion surface in the dead intestine is another factor. When the intestine is allowed to die gradually, as by keeping it under as nearly as possible sterile conditions in oxygenated Tyrode solution, the outward diffusion of glucose proceeds for several hours at a steady rate and then greatly increases as death occurs, as evidenced by the disappearance of movement (Purves(3)). This result cannot, however, be taken as evidence that it was because of death of the epithelium that diffusion became more rapid, for there may have been a change in porosity and also a change in the surface area after the tone was lost. It may be mentioned that changes in filtration pressure were guarded against in all experiments.

There is therefore nothing to show that the retarded diffusion

through living, as compared with dead intestine, is attributable to any other cause than a difference in the physical state of the membrane.

Evidence of the persistence of selective absorption in the surviving loops has however been found in the results obtained in the following experiments: (1) Comparison of rates of diffusion of equimolecular solutions of different sugars; (2) the influence of temperature on the rate of diffusion; (3) the influence of concentration on the rate of diffusion.

(1) With regard to the diffusibility of different sugars Cori and Cori and Cori[8] have shown in the standard white rat that there is a marked difference in the rates of absorption of different sugars from the gastro-intestinal tract, galactose or glucose diffusing much more rapidly than xylose or arabinose. The results of the present investigation show, though less markedly, that the same difference persists in the surviving excised intestine of the rabbit and still more so in that of the cat. These differences were not obtained when the pieces of intestine were previously killed. There can be no explanation for this result other than that the mucous membrane possesses the property of selection.

When the rates of diffusion of equimolecular solutions of the two sugars at different temperatures were compared, it was found that xylose diffused more rapidly than glucose when the temperature was kept near freezing point, but glucose more rapidly than xylose at body temperature.

(2) With regard to the influence of temperature on the rate of diffusion of glucose solutions alone, it has been found that the ratio of increase in the surviving gut between 0° and 20° C. was less than that between 20° and 40° C., whereas in the case of the dead gut, these ratios were much more nearly alike. The conclusion seems justified that something of the nature of selective absorption comes into play as the temperature rises and that this property acts so as to accelerate diffusion. In the light of both these and the experiments with different sugars, as well as those on optimal concentration to be referred to later, we conclude that when the selective factor comes into play it accelerates the rate of absorption for those substances, such as glucose, which are of physiological importance and probably retards that of those which are not required in the organism. If we take xylose as a representative of the latter, then it might be expected that diffusion would become actually less as the temperature was raised, whereas, as our results show, this was not the case, although the rate compared with that for glucose became relatively less. The reason for this result is, no doubt, that the supposedly living intestine in our experiment was really far on the way

to death and, consequently, the physical changes in its walls overweighed the residual vital activity.

(3) Regarding the influence of strength of the inside solution, our results, especially those on the cat intestine, clearly show that the greatest diffusion occurs at a certain concentration which is near to $3M/4$. Stronger solutions than this do not diffuse any more rapidly and, indeed, in several experiments diffused at a slower rate. Weaker solutions than $3M/4$ diffused more or less in proportion to their strengths, and in this particular it has been impossible to detect any significant difference in living as compared with dead intestine. This is no doubt owing to masking of the selective activity by the physical factors. We are entirely at a loss to explain why diffusion should sometimes be less rapid in living segments when the strength of the inside solution rises above $3M/4$. If it merely remained constant for the stronger solutions we might suppose that more molecules of glucose could not pass through than those passing at $3M/4$.

Finally, for those three reasons, namely, (1) that there is a preferential diffusion of glucose over xylose or arabinose; (2) that temperature does not equally affect the rates of diffusion of glucose and xylose, and (3) that there is an optimal concentration at which glucose diffuses most rapidly, we conclude that the intestinal epithelium retains its property of selective absorbability in excised pieces of intestine kept alive in a bath of oxygenated Tyrode solution.

It is not our intention here to review the voluminous literature bearing on our question, but some reference must be made to one or two researches in which absorption from surviving loops of intestine has been studied. The most important of the earlier of these were carried out by Cohnheim(4), who concluded that movement of water occurred through the intestinal walls independently of differences in osmotic pressure. He also found in the isolated intestine of octopods that absorption of sodium iodide occurred unassociated with any movement of water(5). Waymouth Reid(6) also concluded that the isolated intestinal wall could manifest selective properties both towards water and sodium chloride.

Among the more recent papers special attention may be given to that of Mielke(7), in which the conclusion is drawn that no other than purely physical factors come into play in determining diffusion of glucose through the walls of isolated intestinal loops taken either from warm-blooded or cold-blooded animals (rat and carp). This conclusion depends on observations of the changes occurring in the percentage of glucose in the contents of the isolated loops containing hypertonic or hypotonic

solutions of glucose. The loops after being filled with the sugar solution were tied at the two ends and suspended for half an hour in oxygenated Ringer's solution. In the case of the carp, some experiments were done in which the solution was placed in the tied-off intestine left *in situ* and the fish returned to water for a period of 1 hour. In the isolated loops of the rat, it was found (1) that the percentage of glucose became lowered irrespective of whether it had originally been isotonic, hypertonic, or hypotonic, and (2) that this lowering was equally well marked in intestine which had been kept in ice-cold Ringer's solution for 4 hours before being used. There is, however, no evidence to indicate that this chilling would destroy any selective power which the mucosa might possess and, since the power of rhythmic contractility was still preserved, it would certainly require something more substantial than the author's mere statement to justify the conclusion that cold had killed the mucosa. In any case, it is doubtful if observation of changes in percentage of glucose in the lumen contents after a period of half an hour in the experimental conditions chosen, could be expected to reveal any selective absorption.

We are aware that our results do not show all the constancy that might be desired, and we have indicated how some of the causes of this irregularity can be overcome. It will require further modification of the experimental procedure to overcome those as yet unknown influences which, in some cases, cause the property of selective absorption to become so feebly marked that it fails to impress its effects on those due to physical diffusion.

Conclusions.

1. Soluble substances diffuse more rapidly through dead intestinal segments suspended in isotonic saline solutions than through living segments. The difference is due to physical changes in the walls of the segments.

2. The living segments nevertheless exhibit the property of selective absorption, as is shown by the following results: (*a*) Whereas xylose diffuses through dead segments at body temperature more rapidly than glucose, the reverse is the case through living segments. (*b*) At low temperatures in the case of living segments, xylose diffuses more rapidly than glucose. (*c*) The increase of diffusion of glucose through living segments is less marked between 0° and 20° C. than between 20° and 40° C. (*d*) As the strength of the glucose solution is increased, diffusion becomes more rapid up to a certain concentration ($3M/4$), beyond which it remains unchanged or may decline. This optimal concentration is not shown with xylose solutions nor when dead segments are used with glucose.

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THE OXYGEN COMBINING POWER OF MUSCLE HÆMOGLOBIN.

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Introduction.

The identity of muscle hæmoglobin and blood hæmoglobin must still be regarded as an open question, and the same can be said about the function of the first-named substance as an oxygen carrier. Because slight differences between the two substances were always found by spectrophotometric methods, some investigators have come to regard the muscle pigment as a breakdown product of hæmoglobin, while others, among them Whipple and his collaborators(1), accept the identity of muscle hæmoglobin and blood hæmoglobin and attribute the differences to ferment action during the process of extraction. Needham(2) suggests the determination of the oxygen combining power of muscle hæmoglobin as a way out of this difficulty, and to the present writers also the behaviour of the muscle pigment towards oxygen seemed to be of foremost importance for both the above-mentioned questions.

Most of the experiments were done on ox diaphragm; the animal was bled, but the muscle was not transfused and thus still must have contained a small amount of blood. On this muscle the experimental routine was worked out, and afterwards some experiments were conducted on the hind legs of dogs, which after bleeding the animal were transfused from the abdominal aorta with warmed Ringer's solution and massaged at the same time.

Methods.

(*a*) *Preparation of the muscle pulp.* In the course of the experiments the necessity transpired that all manipulations of the tissue should be done at low temperatures. The ordinary procedure, therefore, of grinding the tissue in a meat grinder was disregarded and replaced by freezing the muscle in liquid air and then powdering. The very fine powder was spread on plates and left to thaw.

(*b*) *Preparation of the muscle hæmoglobin.* For our purpose the ordi-

nary extraction methods were of little use, as for accurate determination of the oxygen capacity by gasometric methods a fairly high concentration of the muscle hæmoglobin is required. In a former investigation (3) one of us found muscle tissue when pressed out in a Büchner press to be quite colourless. The resulting fluid, however, showed itself to be both turbid and very viscous and therefore unfit for further preparation. Good results were obtained by centrifuging the thawed-out muscle pulp for half an hour. It is true that part both of the fluid and of the pigment remained in the tissue, but a good deal was pressed out, and on the pigment contained in this solution the determinations were done.

(*c*) *Concentration of the hæmoglobin solution.* The concentration of hæmoglobin, however, in the solution, gained by centrifuging, was not more than one-tenth of the concentration in blood, and therefore had to be concentrated to allow gasometric determinations of sufficient accuracy to be made. On the other hand either warming the solution or even keeping it for long periods at room temperature caused breaking down of the hæmoglobin, and so we had to devise a new method of concentration. The one we finally used was found later to bear a great resemblance to the method of hæmoglobin preparation of Parsons (4) and of Offringa (5). The apparatus consists of two glass tubes *a* and *b*; the smaller one, which is suspended in the other by the ring *c*, having a small hole in its bottom. This opening being closed with a small piece of adhesive tape, tube *a* is filled with the solution to be concentrated and placed in a salt-ice mixture. When the fluid is frozen solid the adhesive tape is taken off, tube *a* inserted in *b* and placed in a centrifuge.

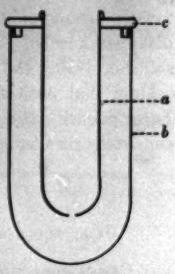

Fig. 1.

In cooling down in the salt-ice mixture, the greater part of the water is first frozen out and afterwards the more concentrated solution. When thawing in the centrifuge, the reverse takes place, and therefore it is possible by stopping the centrifuge at the right moment to get a fairly concentrated solution in tube *b* and nearly clear ice in *a*. The hæmoglobin concentration in the fluid could be raised in this manner to $\frac{1}{4}$ or $\frac{1}{5}$ of the concentration in blood. It was impossible to get it much higher owing to the fact that the concentrated solution proved to be very viscous at low temperatures. Hæmoglobin solution in water, however, can be concentrated by this method to far above blood concentration, and good results were obtained too with other solutions.

(*d*) *Methods of measurement.* After saturating the solution of muscle hæmoglobin with oxygen the amount of oxygen combined was determined by vacuum extraction in the older type of the van Slyke blood gas pump(6). It was at first attempted to determine the concentration of the hæmoglobin in the solution by colorimetry using the reduced hæmoglobin standard solution of Bürker(7). In no way, however, could the colours be made to match, and so this method was replaced by spectrophotometry. Using a Hüffner spectrophotometer the absorption was measured from $\lambda = 554$ to $565\mu\mu$ and from these readings the concentration was calculated.

Discussion.

The oxygen combining power of muscle hæmoglobin could only be demonstrated by taking the precaution of keeping both the muscle pulp and afterwards the solution at low temperatures, and even then the actual gasometric measurements kept somewhat below the values for oxygen as calculated from the spectrophotometric data. This discrepancy may partly be caused by some inaccuracy in the spectrometric measurements due to the slight difference between the spectra of muscle and blood hæmoglobin, but it is more probable that even with the precautions taken the muscle hæmoglobin lost some of its oxygen combining power. A total disappearance of oxygen combining power resulted from keeping the solution during the night even on ice, and for this reason it was very seldom that positive results were obtained in the earlier experiments. Later on, with the experimental routine well developed, we got more consistent results, and from these are taken the following examples:

	Hb g. per 100 c.c.	O_2 per 100 c.c.	
		Found	Calculated
Ox diaphragm (not transfused)	6·1	7·7	8·3
	6·0	7·4	8·0
Dog's muscle (transfused)	3·0	3·2	4·0

Thus we must ascribe to muscle hæmoglobin also the power of combining with oxygen and probably to the same degree as blood hæmoglobin so that both substances can be called identical in this respect. This being the case it must be regarded as possible that the muscle hæmoglobin has a function to perform as an oxygen carrier, and its importance in this respect is enhanced by the fact that, according to Whipple(8), the total muscle hæmoglobin of the body may amount to 80 p.c. of the circulating hæmoglobin.

A transformation of the hæmoglobin to substances which cannot be

made to yield any oxygen occurs probably by ferment action, when one breaks down the tissue structure, as in grinding the muscle. One of these products is methæmoglobin which could be identified by its absorption band for $\lambda = 620\mu\mu$ and by the shifting of this band to the blue end of the spectrum on adding sodium fluoride. The breaking down of hæmoglobin by muscle extract has been demonstrated by Calvo-Criado(9), but our experiments make it improbable that this occurs *in vivo*, as with proper precautions hæmoglobin with nearly full oxygen combining power can be prepared from blood-free muscle tissue.

Summary.

1. A method is described for the preparation of solutions of muscle hæmoglobin up to a concentration of about $\frac{1}{3}$ of the hæmoglobin concentration in blood. This employs a technique for concentrating aqueous solutions of thermolabile substances.

2. The oxygen combining power of muscle hæmoglobin as measured appears to be from 20 to 7 p.c. less than that of blood hæmoglobin; the deficiency we attribute to the necessary loss in the extraction from muscle. We believe the two quantities to be sufficiently close to warrant the assumption that they are identical.

3. Muscle hæmoglobin is easily modified by substances contained in the muscle pulp to products which do not combine with oxygen; one of these is methæmoglobin.

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ALTERATIONS IN THE LACTIC ACID CONTENT OF THE BLOOD AS A RESULT OF LIGHT EXERCISE, AND ASSOCIATED CHANGES IN THE CO_2-COMBINING POWER OF THE BLOOD AND IN THE ALVEOLAR CO_2 PRESSURE.

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I. Introduction.

Many workers have estimated the lactic acid content of the blood of man under different conditions of rest and exercise, and a very brief summary of the main results may first be given.

Lactic acid is now generally accepted as a constant constituent of the blood during rest, but the values given for its concentration by different workers vary greatly. This is probably due to the large variety of methods used for the analysis of the lactic acid, and to the fact that insufficient attention has been paid to the glycolysis in shed blood described by C. Bernard which Evans(1) showed (1922) was associated with a fall in capacity for CO_2. Hill, Long and Lupton(2), using the Clausen technique, and with full precautions against glycolysis, etc., obtained resting values which varied between 10 and 25 mg. lactic acid per 100 c.c. blood. Of this Long(3) concludes, as a result of a comparison between values given by the Clausen method and his own thiophene method, that only one-half to three-quarters represents true lactic acid, the rest being due to other substances erroneously estimated as lactic acid by the Clausen method.

In severe and in moderately severe exercise many have been able to demonstrate the existence of an increased concentration of lactic acid in the blood, including Ryffel(4); Fries(5); Lichtwitz(6); Himwich, Loebel, Barr and Green(7); Hill, Long and Lupton(2); Long(2a); Mendel, Engel, Goldschneider and Bauch(8); Schenk(9); Jervell(10); etc. The associated fall in the CO_2-combining power of the blood (Christiansen, Douglas and Haldane(11); Barr, Himwich and Green(7); Bock, Vancaulaert, etc.(12)), the expulsion of CO_2 from the body during exercise, and its retention again during recovery (Douglas, Haldane, Henderson and Schneider(13); Cook and Pembrey(14); Campbell, Douglas and Hobson(15); MacKeith, Pembrey, etc.(16); Hill, etc.(2)), and the fall in alveolar CO_2 tension (Douglas and Haldane(17)) have all been clearly demonstrated under these conditions.

In the case of moderately severe exercise, which can, however, be maintained for a considerable time, it was evident from the early work on the respiratory exchange and blood bicarbonate, referred to above, that an equilibrium must be attained at an early stage of the exercise between the processes of lactic acid production and removal. This has been verified directly by Hill, Long and Lupton, in their experiments on "lactic acid and muscular exercise"(2). Long(2a) found that for a given degree of exertion the blood lactate increased up to a level which it maintained throughout the exercise, this level being higher the more severe the exercise (see also Jervell(10)). Long found also that the rate of lactic acid oxidation, as measured approximately by the total oxygen uptake of the body at the time, varied as the square of the concentration of lactic acid found in the blood, and this agreed with the work of Hartree and Hill(18) on isolated muscle tissue, in which it was found that the maximal rate of recovery heat production varied as the square of the total initial heat liberated in contraction.

The facts may therefore be accepted as well established for the above conditions, but in marked contrast with this abundance of research carried out on the more severe types of exercise is the scarcity of work performed upon exercise such as walking, which is sufficiently gentle and natural to be considered as normal to everyday life. The only systematic work is that of Long who extended the work mentioned above to cover this type of exercise also. He found that, as is generally assumed, the blood changes differed only quantitatively from the changes in moderate exercise. A lactate increase was still found, and it still agreed roughly with the relation: O_2 uptake α (blood lactate concentration^2). This would

imply that even in very light exercise, for which the body is presumably as fully adapted as possible, a generalized increase in lactate occurs in the body, and the task of lactate oxidation and resynthesis is distributed throughout the entire body and not confined to the active tissues alone. The resting lactate concentration would thus probably correspond to the metabolism of the heart and respiratory muscles which occurs in rest.

The following results are collected from the papers of Hill, Long and Lupton, and Long, and are given for comparison with results obtained in the present research. They are corrected where necessary to bring them all to terms of lactate in whole blood:

TABLE I.

Subject	Rate of walk (m.p.h.)	Duration of exercise (min.)	Lactic acid (mg.) per 100 c.c. blood		
			Before walk	After walk*	Increase
J. C. H.	3·3	20	21·5	30·1	8·6
J. C. H.	3·3	30	13·6	31·3	17·7
J. C. H.	3·3	25	21·0	27·1	6·1
C. N. H. L.	3·5	28	13·9	24·4	10·5
C. N. H. L.	3·5	30	12·2	28·1	15·9
C. N. H. L.	3·5	30	14·0	29·6	15·6
S. S.	3·5	30	15·7	31·5	15·8
K. F.	3·7	30	11·6	28·3	16·7
C. N. H. L.	4·1	33	14·3	39·3	25·0
C. N. H. L.	4·1	30	10·1	31·3	21·2
C. N. H. L.	4·1	30	14·3	52·0	37·7
P. E.	4·5	25	12·2	30·1	17·9

* The sample after the walk was taken 1 min. from cessation of the exercise.

In the above experiments no determinations were made of the CO_2-combining power of the blood, nor of the alveolar air changes.

In their early work on similar light exercise Douglas, Haldane and Priestley (17, 19) considered the increase found in the alveolar CO_2 tension to be adequate to account for the increase in respiration which occurred at the same time, and this does not agree with the above results. There is, however, a possibility that the error, which must be introduced into such determinations as a result of the increased importance of the delay involved in making a forced expiration when the breathing is augmented by exercise (Krogh (20)), was sufficient to mask the real state of affairs. Dr Douglas and the writer (21) therefore attempted to demonstrate, following walking at 3 m.p.h. for an hour, the fall of CO_2-combining power of the blood which would be expected to accompany changes of blood lactate of the magnitude of those found by Long. In two experiments no success was obtained, there seeming to be a tendency for the bicarbonate to increase rather than fall.

More striking, however, is the experiment of Bock, Vancaulaert,

etc.(12), on de Mar, a Marathon runner of unusual ability and training. This subject was found to show as a result of a 15 mile run a rise of only about 10 mg. p.c. lactate in the blood, and this was controlled by estimations of the CO_2-combining power of the blood. Towards the end of the run the total pulmonary ventilation was measured as 90·7 l. per min. and the oxygen uptake as 3·5 l. per min., these values being the mean of two separate determinations. Bearing in mind that this is a metabolic level which many would find beyond their powers to maintain at all, this would seem to differ fundamentally from the results of Hill, who looks upon the generalized lactate increase in the body as part of the normal mechanism of increased removal.

In view of these and other scattered observations it seemed necessary to obtain further data upon the reaction of the body to light exercise, such as walking, and it was in the hope of obtaining these that the present research was undertaken.

II. Experimental methods.

Blood samples were in all cases taken without stasis from convenient veins of the forearm, through a hollow needle into a 20 c.c. syringe, a known amount of potassium oxalate having been first placed in the latter. The syringe was inverted several times, until the oxalate was carried into the blood, and the latter was then expelled into a Pyrex-glass tube, containing a known amount of sodium fluoride. The blood was well mixed, and the tube placed on ice, and only removed when some of the blood was needed for analysis. The volume of blood removed from a vein in one sample was kept as near 15 c.c. as possible. In the earlier experiments 0·06 g. oxalate and 0·015 g. fluoride were used, but in the later experiments these amounts were reduced to 0·03 g. and 0·005 g. respectively, the latter corresponding to 0·2 p.c. oxalate and 0·03 p.c. fluoride approximately.

Bicarbonate analyses were performed by the latest form of the Haldane ferricyanide method(22), and the apparatus was completely sunk in an electrically controlled thermostat, at 20° C. All the specimens of sodium fluoride available in the laboratory proved to be sufficiently acid in solution to cause serious errors in the bicarbonate analyses. This acidity was presumably due to the presence of a certain amount of the double salt NaF.HF, and was overcome by heating the salt to red heat, when the HF would be driven off. A few grams of fluoride were treated in this way at the beginning of the research, and after grinding as finely as possible were stored in a well-stoppered bottle for use throughout the

research. Repeated tests showed no deterioration of this sample. This is mentioned, since it is possible that significant errors may be introduced into similar work unless this factor be carefully controlled. The heated fluoride was very slightly alkaline in solution, but much too slightly to cause any error, granted that moderately even solution were obtained in the blood. It was found necessary to shake up the blood very thoroughly before putting it on ice so as to get all the fluoride and oxalate into solution, as otherwise much of the solid would tend to be taken up in the pipette while removing blood from the main tube to perform one bicarbonate analysis. If this occurred the analysis gave a higher value for the CO_2-combining power than subsequent duplicates which agreed together, and controls seemed to show a bigger effect than would be expected from the alkalinity of the fluoride alone. Possibly some other salt action occurs. This error was eliminated by reducing the amounts of oxalate and fluoride used to the lower limits given, and by thorough shaking of the tube as described, but it is another possible source of error which must always be kept in mind, especially if duplicates are not performed. In the present work duplicates were always performed, and at sufficient time apart to show up any progressive change which might be occurring in the blood.

Lactate analyses. The method of Friedemann, Cotonio and Shaffer(23) was used, without modification from the published procedure, except that in order to obtain the accuracy required it was found necessary to use glass-blown apparatus, so as to eliminate the rubber bung from the reaction vessel. All analyses were carried out in duplicate on two sets of apparatus, and the duplicates normally agreed to within 0·02 c.c. *N*/100 iodine, and always to within 0·04 c.c. Protein precipitation was performed by trichloracetic acid. The sample of this used at first gave no blank, but later samples all gave a small blank (about 0·1 c.c. *N*/100 iodine) which was quite constant for a given solution of acid. Unfortunately this was not noticed for some little time, and therefore the lactate values of the preliminary experiments are not accurate to a milligram or two per 100 c.c. blood, though relatively in any experiment they are accurate. The copper-lime method of van Slyke(24) was used for sugar removal. The actual procedure was as follows—5 c.c. blood were run slowly into 25 c.c. 7 p.c. CCl_3COOH in a centrifuge tube. The latter was closed by a rubber bung, and the contents well shaken. After standing an hour or so the mixture was centrifuged, and 25 c.c. of clear fluid obtained. To this 5 c.c. 10 p.c. $CuSO_4$ solution, followed by 20 c.c. 5 p.c. lime suspension, were added, and the mixture

shaken mechanically for ½ to 1½ hours, when it was centrifuged. Enough clear fluid was obtained to perform two analyses on 20 c.c. each. Full blanks were performed in all later experiments.

Phosphate analyses. The method of Fiske and Subbarow was used (25).

Hæmoglobin values were obtained by means of the Gowers-Haldane hæmoglobinometer.

III. Control experiments.

Since the research depended upon the accuracy of the lactate analyses a considerable number of control experiments were performed, from which the following are taken at random:

Table II. Analyses of pure zinc lactate solutions.

Lactate analysed— in lactic acid (mg.)	Lactic acid found (mg.)	Recovery (p.c.)
0·286	0·284	99
0·572	0·579	101
0·858	0·855	100
0·509	0·550	108
0·467	0·490	105
0·934	0·980	105
0·464	0·475	102
0·928	0·933	101
0·464	0·460	99
0·940	0·928	99
0·940	0·911	98
0·962	0·942	97

The lactate used in the above gave 99·7 p.c., the theoretical weight of ash, on heating.

Table III. Recovery from blood of added lactate.

	Lactate added, as lactic acid (mg. p.c.)	Lactic acid found (mg. p.c.)	Lactic acid increase (mg. p.c.)	Recovery (p.c.)
(*a*)	0·0	14·7	—	—
	7·6	22·7	8·0	105
	15·1	30·5	15·8	105
(*b*)	0·0	38·9	—	—
	19·1	57·5	18·6	97
(*c*)	0·0	46·6	—	—
	10·1	55·6	9·0	89
	15·1	61·1	14·5	96
	20·1	65·8	19·2	96

It may be pointed out that in the above, since slaughter-house blood was used, the total amounts analysed were greater than in the actual experiments, where the average was about 12 mg. lactic acid p.c., and this would increase the errors. In spite of this there is a general accuracy well within 1 mg. p.c. The comparative accuracy of the method is

better shown by the results of duplicate extractions upon fresh human blood. The following are all the cases in which this double extraction was performed in the normal course of experiments, and the absolute agreement shows that there was a relative accuracy to within the limits of the titration, *i.e.* one drop of iodine, or about 0·5 mg. lactic acid p.c.:

TABLE IV. Duplicate extractions and analyses on blood lactate.

Lactic acid of blood (mg. p.c.)	
A	*B*
14·7	14·9
9·8	9·8
12·3	12·3
14·9	14·9
14·7	14·7

Controls were also carried out, in which 5 c.c. of diluted blood as well as 5 c.c. of undiluted blood were extracted, and—as the values obtained agreed well—this controlled the accuracy of the blank values obtained, and the efficiency of the methods of extraction.

In performing the phosphate analyses the standard time of 10 min. between mixing the reagents and colour comparison was often extended to some hours. The following controls show the general accuracy of the method, and the fact that the above delay had no significant effect on relative values:

TABLE V. Control experiments for analyses of inorganic phosphate.

Phosphate analysed, as phosphorus (mg.)	Phosphorus found (mg.), with colour comparison	
	At 15 min.	At 12 hours
0·160	0·160	0·160
0·160	—	0·160
0·200	0·200	0·195
0·180	—	0·178
Blood (*a*)	2·92 p.c.	3·10 p.c.
„ +0·4 p.c.	3·32 p.c. (+0·39)	3·51 p.c. (+0·41)
Blood (*b*)	3·38 p.c.	3·45 p.c.
„ +added phosphate	3·62 p.c. (+0·24)	3·67 p.c. (+0·22)

IV. EXPERIMENTAL RESULTS.

The greater number of experiments were performed on the following general plan. The experiment was carried out between about 9 a.m. and 11.30 a.m., and the subject had then had no food since the previous evening. He first rested for an hour in a deck-chair in the laboratory, and at the end of this period—with as little disturbance as possible—one arm was immersed in some hot water for a few minutes and a blood sample collected from a convenient vein. The object of the hot water

was to increase the blood flow through the arm, and so to render the blood obtained more arterial in character. The subject next walked round a track measured out outside the laboratory (about seven laps to a mile) for about 30 min., this being considered long enough to permit of the establishment of fairly complete equilibrium throughout the body.

If an alteration in the lactate concentration occurred of the order of that found by Long, and if equilibrium had been established throughout the body, it was not considered likely that all this lactate could be removed very rapidly after the exercise, so that time was allowed in the earlier experiments to immerse the arm for a short time in hot water before taking the second sample. This therefore was not obtained for 3 to 5 min. after cessation of the walk.

The results of the preliminary experiments are given below:

TABLE VI. Preliminary experiments.

Subject	Rate of walk and duration	Blood lactate found (mg. p.c.)		Combined CO_2 in c.c. p.c. at S.T.P. and CO_2 pressure in saturator
W. H. O.	4 m.p.h. for 38 min.	After 1 hr. rest	14·5	48·7 (at 41·0 mm. Hg)
		4½ min. after walk	13·5	48·7 (at 40·7 „)
W. H. O.	4 m.p.h. for 45 min.	After 1 hr. rest	20·5	— —
		4½ min. after walk	21·3	— —
W. H. O.	4·2 m.p.h. for 47 min.	After 1 hr. rest	11·5	48·2 (at 41·9 „)
		4 min. after walk	11·8	49·9 (at 41·8 „)
W. H. O.	4·5 m.p.h. for 30 min.	After 1 hr. rest	—	45·5 (at 39·7 „)
		After 1 hr. rest	14·5*	44·6 (at 39·5 „)
		5 min. after walk	16·4*	45·4 (at 39·7 „)
C. G. D.	4·0 m.p.h. for 60 min.	After 1 hr. rest	14·5	46·1 (at 38·9 „)
		3 min. after walk	13·9	46·6 (at 39·2 „)
C. G. D.	4·9 m.p.h.	After 1 hr. rest	16·0	46·5 (at 44·3 „)
		5 min. after walk	15·3	46·7 (at 44·3 „)

* Poor lactate analyses in this case.

Since the above six experiments were performed at an early stage of the research, in which CCl_3COOH blanks were not done, these values may be 1 or 2 mg. p.c. too high, but are relatively correct.

Since these experiments showed no increase in blood lactate, nor any fall of CO_2-combining power of the blood after exercise, it was desirable to obtain blood samples from the subjects actually during the course of the exercise. No mechanical platform being available this proved impracticable for walking exercise, and it was necessary to compromise, obtaining blood samples as soon as possible after the cessation of a walk, and in parallel experiments obtaining blood samples actually during the course of light exercise on a cycle ergometer.

Taking first the walk experiments, it was impossible, if the sample were

to be obtained rapidly after cessation of exercise, to submerge the arm in hot water before taking this sample. It is not thought that this can make any real difference to the results, since the exercise itself almost always sufficed to put up the circulation rate in the arms considerably, especially if the hands were kept covered during the walk. On cold days, however, this increase in local circulation rate did not occur, with the result that the attempt to obtain the blood sample rapidly after cessation of exercise failed. This fact is really fortunate in that it provides a control on the experiments—if the blood flow in the arm were not increased as a result of the exercise, and the conditions before exercise and using hot water, and after exercise without the use of hot water, were not strictly comparable the experiment was automatically eliminated. The prominence of the arm veins may be taken as a rough criterion of the local blood flow, and it was found that they normally appeared of similar sizes in the

TABLE VII. Experiments on walking exercise, subject W. H. O.

	Rate of walk and duration	Blood lactate found (mg. p.c.)		Combined CO_2 in c.c. p.c. at S.T.P. and CO_2 pressure in saturator
1.	3·9 m.p.h. for 30 min.	After 1 hr. rest	14·9	— —
		20–80 sec. after walk	14·7	— —
2.	4·0 m.p.h. for 30 min.	After 1 hr. rest	10·0	48·5 (at 39·8 mm. Hg)
		25–60 sec. after walk	7·5	48·5 (at 40·5 „)
		Do. (repeat at 24 hrs.)	9·3	— —
3.	4·2 m.p.h. for 28 min.*	After 1 hr. rest	10·6	47·7 (at 40·0 „)
		20–70 sec. after walk	11·1	48·6 (at 40·3 „)
4.	4·5 m.p.h. for 32 min.†	After 1 hr. rest	8·4	48·6 (at 45·1 „)
		30–90 sec. after walk	13·1	47·2 (at 45·2 „)
5.	4·5 m.p.h. for 27 min.	After 1 hr. rest	14·8	— —
		27–† sec. after walk	14·8	— —
6.	4·5 m.p.h. for 25 min.	After 1 hr. rest	12·2	47·3 (at 42·3 „)
		25–55 sec. after walk	12·0	46·4 (at 42·5 „)
7.	4·5 m.p.h. for 27 and 26 min., 5 min. rest between	After 1 hr. rest	11·2	48·4 (at 42·3 „)
		35–75 sec. after walk	11·2	48·0 (at 42·4 „)
8.	4·7 m.p.h. for 25 min.	After 1 hr. rest	11·4	47·0 (at 40·9 „)
		30–68 sec. after walk	19·3	44·7 (at 42·0 „)
9.	4·8 m.p.h. for 26 min.	After 1 hr. rest	12·0	47·1 (at 41·1 „)
		25–55 sec. after walk	24·7	44·0 (at 41·6 „)
10.	5·0 m.p.h. for 40 min.	After 1 hr. rest	10·4	— —
		c. 30 sec. after walk	50·4	— —
11.	5·4 m.p.h. for 14 min.	After 1 hr. rest	7·3	46·2 (at 40·4 „)
		20–80 sec. after walk	53·1	38·0 (at 40·1 „)

* The walk was carried out in this experiment before the rest period.
† No CCl_3COOH blanks were done in this experiment.

Walking at 4·0 m.p.h.: O_2 consumption = 1·41 l. per min. at S.T.P.
„ 4·3 „ „ = 1·50 „ „
„ 4·8 „ „ = 1·92 „ „
„ 4·9 „ „ = 1·95 „ „

above two conditions. Light pressure was applied to the veins by the subject until the needle had been introduced into the vein, but none during the taking of the sample. The actual procedure was that during the last moments of the walk the subject removed his jacket, and bared the arm from which the sample was to be taken. He then sat down on a stool and at the same time started a stop watch. The sample of blood was taken as quickly as possible, and the time limits noted on the watch. Usually the sample was obtained between about 20 sec. and 1 min. after cessation of the walk. The results are given in Tables VII and VIII,

TABLE VIII. Experiments on walking exercise, subject C. G. D.

	Rate of walk and duration	Blood lactate found (mg. p.c.)		Combined CO_2 in c.c. p.c. at S.T.P. and CO_2 pressure in saturator
1.	4·0 m.p.h. for 31 min.*	After 1 hr. rest 15–60 sec. after walk	10·6 18·9	47·4 (at 40·5 mm. Hg) 46·5 (at 39·3 „)
2.	4·0 m.p.h. for 30 min.*	After 1 hr. rest 15–60 sec. after walk	13·1 8·0	45·0 (at 39·9 „) 46·3 (at 39·0 „)
3.	4·0 m.p.h. for 30 min.	After 1 hr. rest 25–60 sec. after walk	8·0 7·1	— —
4.	4·5 m.p.h. for 26 min.	After 1 hr. rest 20–50 sec. after walk	9·9 10·7	47·3 (at 42·4 „) 47·2 (at 41·6 „)
5.	4·5 m.p.h. for 28 and 15 min., 3 min. between	After 1 hr. rest 25–55 sec. after walk	10·6 10·3	49·2 (at 40·6 „) 48·1 (at 41·2 „)
6.	4·7 m.p.h. for 30 min.	After 1 hr. rest 20–60 sec. after walk	9·8 12·3	47·5 (at 39·2 „) 47·5 (at 40·1 „)
7.	4·9 m.p.h. for 31 min.†	After 1 hr. rest 15–60 sec. after walk	9·1 13·7	46·6 (at 41·0 „) 47·1 (at 40·5 „)
8.	4·9 m.p.h. for 28 and 15 min., 4 min. between	After 1 hr. rest 15–47 sec. after walk	8·7 12·6	47·5 (at 40·8 „) 48·7 (at 41·2 „)
9.	4·9 m.p.h. for 33 min.	After 1 hr. rest 20–50 sec. after walk	17·6 21·0	46·9 (at 42·3 „) 47·2 (at 41·4 „)

* The walk was carried out in this experiment before the rest period.
† No CCl_3COOH blanks were done in this experiment.

Walking at 4·0 m.p.h.: O_2 consumption = 1·36 l. per min. at S.T.P.
„ 4·5 „ „ = 1·85 „ „
„ 5·0 „ „ = 2·28 „ „

which also contain the values for O_2 consumption obtained in parallel experiments on the same subject.

The ergometer experiments were undertaken since this was the only type of exercise available during which it was possible to take blood samples without any interruption of the exercise. A Krogh electric brake ergometer was used, and in all the experiments the same rate of work was adopted, *i.e.* 525 kg. m. per min., with a rate of rotation of the pedals of 50 per min. This level of exercise was chosen as giving an oxygen

consumption comparable to that during walking exercise at 4·0 m.p.h. (*c.* 1·4 l. O_2 per min.).

The procedure was similar to that in the walk experiments. The subject, who was always in the post-absorptive state at the time, rested in a deck-chair for an hour as before. At the end of the hour a blood sample was collected, the arm having been warmed up first in hot water. The subject then commenced work on the ergometer, and after a convenient tim a second blood sample was collected without interruption of the work. In this case also it was possible to warm up the arm first, by placing it in a bucket supported at the side of the ergometer. Thus the work sample was in every way comparable to the rest sample. About 5 min. after the second sample had been taken the subject ceased work, and at once got off the ergometer and sat down again in the chair, at the same time starting a stop watch. A third blood sample was then collected, without the use of hot water, the time limits being again noted on the watch.

The results are given in Table IX:

TABLE IX. Ergometer experiments at 525 kg. m. per min.

	Subject	Blood lactate found (mg. p.c.)		Combined CO_2 in c.c. p.c. at s.t.p. and CO_2 pressure in saturator
1.	W. H. O.	After 1 hr. rest	10·3	48·0 (at 41·6 mm. Hg)
		During work (at 40 min.)	13·4	46·4 (at 42·3 „)
		1½–3½ min. after work	11·8	47·3 (at 41·9 „)
2.	W. H. O.	After 1 hr. rest	8·2	— —
		During work (at 55 min.)	8·2	— —
		During work (at 68 min.)	12·7	— —
		3–4 min. after work	11·9	— —
3.	W. H. O.	After 1 hr. rest	12·3	46·0 (at 42·9 „)
		During work (at 25 min.)	11·1	47·0 (at 42·1 „)
		4½–5½ min. after work	10·5	47·0 (at 42·1 „)
4.	C. G. D.	After 1 hr. rest	11·0	47·5 (at 40·8 „)
		During work (at 28 min.)	25·7	45·4 (at 41·6 „)
5.	C. G. D.	After 1 hr. rest	16·0	42·2 (at 40·8 „)
		During work (at 30 min.)	25·5	41·4 (at 40·8 „)
		2½–4 min. after work	23·9	40·9 (at 41·0 „)
6.	C. G. D.	After 1 hr. rest	11·1	46·5 (at 42·4 „)
		During work (at 44 min.)	16·7	45·9 (at 42·0 „)
		6–6½ min. after work	13·6	46·1 (at 42·0 „)
7.	C. G. D.	After 1 hr. rest	11·6	48·8 (at 42·4 „)
		During work (at 32 min.)	19·3	47·1 (at 41·6 „)
		5½–6½ min. after work	18·6	47·4 (at 41·8 „)

W. H. O. working as above: O_2 consumption = 1·40 l. per min. at s.t.p.
„ „ „ „ = 1·32 „
C. G. D. „ „ „ = 1·43 „
„ „ „ „ = 1·40 „

Since the CO_2-combining power of the blood might be affected by changes in dilution, or in the inorganic phosphate content during exercise, the hæmoglobin values of the blood samples were taken in all cases, and the inorganic phosphate content estimated in the earlier experiments. The hæmoglobin values showed no constant or significant alterations, and need not be given here. The phosphate values obtained are given in Table X:

TABLE X. Inorganic blood phosphate changes during exercise.

Subject	Exercise	Inorganic phosphorus (mg. p.c.) Before	After	Change	Lactate change (mg. p.c.)
W. H. O.	Walk at 4·0 m.p.h. for 38 min.	2·86	3·02	+0·16	− 1·0
,,	,, 4·2 ,, 47 ,,	3·24	3·51	+0·27	0
,,	,, 4·5 ,, 30 ,,	3·72	3·75	+0·03	0
,,	,, 4·5 ,, 30 ,,	3·70	3·98	+0·28	0
,,	,, 4·5 ,, 32 ,,	3·68	3·87	+0·19	+ 4·7
C. G. D.	,, 4·0 ,, 61 ,,	3·44	4·00	+0·56	—
,,	,, 4·0 ,, 60 ,,	3·38	3·62	+0·24	+ 0·6
,,	,, 4·9 ,, 32 ,,	3·67	3·90	+0·23	+ 0·7
W. H. O.	Ergometer work for 45 ,,	3·98	4·57	+0·59	+ 3·1
C. G. D.	,, ,, 30 ,,	4·02	4·24*	+0·22	+14·7
,,	,, ,, 30 ,,	4·20*	3·99	+0·21	+ 1·6

The after-work samples were in most cases obtained about 45 sec. after the end of the walk, 2 min. after the end of the ergometer work.

Ergometer work was 525 kg. m. per min. on Krogh electric cycle ergometer.

* Sample taken during work.

Some determinations were made in the usual manner of the oxygen debts incurred in exercise such as studied in the foregoing experiments. The results need not be given in full, but the estimated O_2 debts were as follows:

TABLE XI. Oxygen debts following exercise.

Subject	Exercise	O_2 debt, in l. at S.T.P.
W. H. O.	Walk at 4·0 m.p.h. for 30 min.	1·09
,,	,, 4·5 ,, 30 ,,	0·99
,,	Ergometer work for 30 ,,	0·97
C. G. D.	Walk at 4·0 m.p.h. for 30 ,,	1·07
,,	,, 4·5 ,, 30 ,,	1·49
,,	Ergometer work for 30 ,,	1·62

Ergometer work was 525 kg. m. per min. on Krogh electric ergometer.

A certain number of determinations were made of the alveolar CO_2 pressure changes before and after exercise of the type studied throughout this work. The results obtained are given graphically, as they are most intelligible in this form.

A comparison has been made between the lactate values given by the Clausen[26] and Friedemann[23] methods of analysis, and the results are given in Tables XII and XIII.

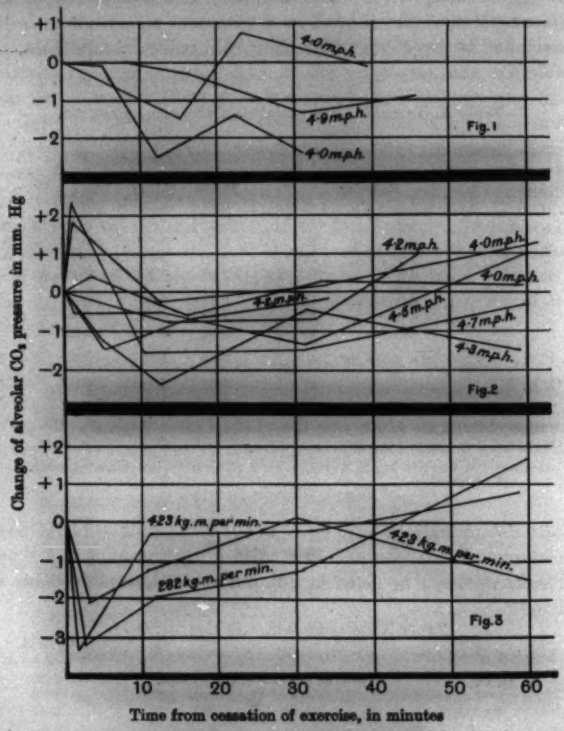

Fig. 1. Subject C. G. D., walking exercise.
Fig. 2. Subject W. H. O., walking exercise.
Fig. 3. Subject W. H. O., ergometer exercise.

It is customary to multiply the value obtained by the Clausen technique by some factor, in order to allow for the low recovery in analyses of pure lactate solutions. The factor adopted in the third column below (*i.e.* × 100/90) is as low as any used in the published work,

and seems near enough for the present purposes, judging from the three analyses given in Table XII, without further justification.

TABLE XII. Comparison of Clausen and Friedemann methods of lactate analysis applied to pure lactate solutions.

Lactate analysed as lactic acid (mg.)	Friedemann value. Lactic acid (mg.)	Clausen value. Lactic acid (mg.)
0·572	0·579	0·545 (95 p.c.)
0·858	0·855	0·727 (85 p.c.)
0·464	0·460	—
0·928 (same sample as next above)		0·853 (92 p.c.)

TABLE XIII. Comparison of Clausen and Friedemann methods of lactate analysis applied to blood extracts.

	Friedemann value (mg.)	Clausen value (mg.)	Clausen value ×100/90 (mg.)
Slaughter-house blood	28·1	36·0	40·0
W. H. O. casual sample	14·8	19·2	21·3
Do. veins compressed	15·2	28·0	31·1
C. G. D. resting sample	9·0	7·7	8·6
Slaughter-house blood	15·5	18·1	20·1
Do. extracting less blood	15·9	18·7	20·7
Slaughter-house blood	38·9	43·0	47·9
Do. +19·1 mg. p.c.	57·5	59·3	65·9
C. G. D. resting sample*	9·8	10·9	12·2
Do. after walk at 4·7 m.p.h.*	12·3	15·7	17·5
W. H. O. resting sample*	14·9	14·7	16·3
Do. after walk at 3·9 m.p.h.*	14·7	14·5	16·1

* Values of this experiment are the means of duplicate extractions and analyses, which agreed well together.

V. DISCUSSION OF RESULTS.

Before discussing the actual results obtained it must be pointed out that the experiments were performed throughout on the same two subjects, C. G. D. and W. H. O. These subjects differ markedly both in age and in build, so that it is considered as improbable that any results obtained on both can have been due to individual idiosyncracies. Both subjects were fit, but in no sense in athletic training, most of their time being spent in ordinary laboratory work, with occasional games of golf and tennis. Thus they may be taken as corresponding physically to average active adults.

Resting blood lactate concentrations. The values obtained for the samples of blood taken at the end of 1 hr. rest are given below, being retabulated from the tables already given. Only those values are included which are considered to be correct absolutely (*i.e.* when full blanks were performed):

TABLE XIV. Lactic acid of blood during rest.

	Subject W. H. O. Lactic acid (mg. p.c.)	Subject C. G. D. Lactic acid (mg. p.c.)
	10·3	11·0
	8·2	16·0
	12·3	11·1
	14·8	11·6
	12·2	9·9
	7·3	8·7
	11·2	10·6
	10·8	17·6
	11·9	10·6
	11·4	13·1
	10·6	8·0
	10·0	9·8
	14·9	—
Average	11·2	11·5

The resting lactate values obtained seem to be lower and somewhat less variable than most in the literature (*v.* Table I for values obtained by Hill, Long and Lupton). The most probable explanation of this fact would be that the Friedemann method here used gives lower values on blood extracts than the various methods used by former workers. The best of earlier methods was probably the so-called Clausen method (26), and this was used by Hill, etc., in their work. In Tables XII and XIII have been given the results of experiments in which the Clausen and Friedemann methods have been compared, when applied to solutions of pure lactate and also to blood extracts. From these it is clear that the Clausen method must estimate as lactic acid other substances present in the blood, since the Friedemann method gives higher values on pure lactate solutions, and gives an almost 100 p.c. recovery of added lactate in blood, but none the less gives lower absolute values on blood extracts. It is of course impossible to say how far the Friedemann values represent true lactic acid, but they would seem certainly to be nearer to the truth than those given by the Clausen method.

Lactate changes during light exercise. It can be seen from the tables of results given that in most cases parallel analyses were performed on the lactate content and CO_2-combining power of the blood samples obtained. The object of the latter was to provide a control on the lactate changes, if any, found by the direct analyses, but for this control to have any value it was necessary to be reasonably certain that no significant alteration occurred in the CO_2-combining power of the blood from other causes than lactic acid changes. Dodds (27) has shown that the secretion of the digestive juices during digestion is accompanied by marked altera-

tions in the acid-base equilibrium of the body, so that in order to avoid this the subjects were always in the post-absorptive state at the time of an experiment. Even with this precaution it is possible that if an experiment be allowed to overlap, or even approach, the normal meal times some error may arise from the same cause, so that experiments were performed as far as possible at periods intermediate between meal hours. Thus if the experiment commenced at 9 a.m. the rest period ended at about 10 a.m. and the blood samples were collected between 10 a.m. and 11.30 a.m. In this way it is hoped that this error was eliminated. Again, changes in dilution of the blood were controlled by means of the hæmoglobin values of the blood samples, and as already stated no significant alterations found. Changes in the complicated buffer substances of the blood could not be fully controlled, but the inorganic phosphate content of the blood was determined in several early experiments (Table X), and no changes of importance from this point of view were found. Changes in acetone bodies were not properly controlled, but no investigators have found any important changes following ordinary light exercise, nor was any increase found in acetoacetic acid or free acetone in a few cases tested in the present work (these observations were made by placing bisulphite in the receiving vessels of the Friedemann apparatus during the preliminary boiling period, before addition of $KMnO_4$, when any acetone—either preformed or produced by hydrolysis of acetoacetic acid in the acid contents of the reaction vessel—should be trapped, and capable of estimation in the usual manner). In view of all this it seems justifiable to correlate the lactate and bicarbonate changes found.

Turning then to the actual results obtained, it is clear from Table VI that the preliminary experiments gave evidence neither of an increase in blood lactate following exercise, nor of a fall in the CO_2-combining power of the blood. The rates of walk varied between 4 and 4·5 m.p.h. for W. H. O. and 4 and 4·9 m.p.h. for C. G. D., and the after-work samples were obtained between 3 and 5 min. from cessation of the exercise. The duration of the walk varied between half and one hour, and must have been sufficient to allow of the establishment of equilibrium throughout the body, and it was considered unlikely therefore that if a generalized lactate increase had occurred of the order of that found by Hill, etc., it could have been completely masked within so short a time. This assumption has since been verified during the course of the ergometer experiments, to be discussed later. From the present point of view the following results may be quoted from the latter experiments

(Table IX): on W. H. O. (Exp. 2), an increase of 4·5 mg. p.c. lactate during work had only decreased to 3·7 mg. p.c. 3½ min. later; on C. G. D. (Exp. 5), an increase of 9·5 mg. p.c. during work had fallen to one of 7·9 mg. p.c. 3½ min. later; on C. G. D. (Exp. 6), an increase of 5·6 mg. p.c. had fallen to one of 2·5 mg. p.c. 6½ min. later; on C. G. D. (Exp. 7), an increase of 7·7 mg. p.c. had fallen to 7·0 mg. p.c. 6 min. later; and against this there were no experiments in which an appreciable lactate increase had been masked within 5 min. from cessation of exercise.

In view of the later experiments (Tables VII and VIII) in which the after-walk sample was obtained between the limits of about 20 sec. and 60 sec. after exercise, these early experiments need not be discussed further now, for in so short a time it is almost inconceivable that a significant lactate alteration in the blood could have been masked, granted that approximate equilibrium had been established in the body generally. Hill, Long and Lupton state that the work samples in their experiments were taken 1 min. after cessation of the walk, but do not specify if this was the time of the beginning or end of the sample, or the mean time of these two, but in any case the samples in the present work must have been obtained at least as rapidly.

In spite of the speed with which the after-work samples were obtained, there is only one experiment on either subject (C. G. D., Table VIII, Exp. 1) in which any increase in lactate concentration was found in the blood at rates of walking below 4·5 m.p.h., and at 4·0 m.p.h. there seems some tendency towards an actual fall in blood lactate.

Of the four experiments on W. H. O. at 4·5 m.p.h., and of the two on C. G. D. at the same rate of walking, only the first on W. H. O. showed any increase in the lactate concentration in the blood, and the increase there was only 4·7 mg. p.c. In this one case showing a rise it is probably significant that the subject was feeling rather unwell at the time, and was quite fatigued at the end of half an hour's walk, even at the easy pace studied. Between 4·5 m.p.h. and 4·7 m.p.h. there seems to be, for both subjects, some critical rate of walk, above which only did an increase in blood lactate follow the exercise. In the case of W. H. O., who is of smaller build than C. G. D., the effort of walking at rates above 4·75 m.p.h. is greater than it is for C. G. D., and it also increases more rapidly as the rate is put up. Probably C. G. D. walks normally at a rate slightly above that adopted by W. H. O. In accordance with these facts the lactate increase found in the blood at rates over the critical speed rises more rapidly in W. H. O. than in C. G. D.

The reason for the one discordant result on C. G. D. already mentioned, where an increase of 8·3 mg. p.c. lactic acid was found following a walk at 4·0 m.p.h., is quite unknown, as the experiment seemed in every detail identical with the others. It may be of some significance that this was the next experiment on C. G. D., though at an interval of 14 days, to that in which he gave the highest resting lactic acid level, 17·6 mg. p.c.

The bicarbonate analyses control the results obtained by the direct lactate analyses very well. Thus for W. H. O. in Exps. 2, 3, 6, 7, no increase was found in the blood lactate, and for these experiments the average of the bicarbonate analyses gives:

Before walk: 48·0 c.c. p.c. CO_2 at 41·1 mm. Hg CO_2 pressure in saturator.
After „ 47·9 „ 41·4 „ „

In all the other experiments on W. H. O. an increase was found in the blood lactate, and this was in every case accompanied by a fall in the CO_2-combining power of the blood, which corresponded in magnitude to the lactate rise, and was very evident with any appreciable lactate alteration.

In the case of C. G. D. none of the experiments were at fast enough rates to give, in this subject, a marked alteration of blood lactate, and in accordance with this there was in no case found a significant fall of CO_2-combining power of the blood. In Table VIII, Exps. 7, 8 and 9, the changes are not in agreement, but as they are small this is of no real importance, and on the whole the lactate and bicarbonate changes agree together extremely well in both subjects.

Lactate changes in light ergometer exercise. The results of the ergometer experiments are interesting from several points of view, especially since in them the blood samples were taken actually during the course of the exercise. In the case of C. G. D., who had not done any bicycling for several years, the contrast between the effects of this type of exercise, and those of walking exercise, is very striking. Thus in the first experiment of this type on C. G. D. the blood lactate increased from a normal rest value of 11·0 mg. p.c. to 25·7 mg. p.c. during exercise, although the severity of the exercise, as measured by O_2 uptake, only corresponded to that of a 4 m.p.h. walk. In successive experiments the lactate rise found tended to diminish, and was in order—14·7 mg. p.c., 9·5 mg. p.c., 5·6 mg. p.c., and 7·7 mg. p.c. This was clearly an example of the effect of training, or adaptation of the body to the particular exercise. W. H. O., in contrast to C. G. D., had carried through the experiments on alveolar CO_2 changes

which involved the use of the ergometer, and had in the interval between then and the present experiments done some cycling, and in his case the lactate rise found was always small or zero. In successive cases it was 3·1 mg. p.c., 0 mg. p.c. at 55 min. work, and 4·5 mg. p.c. at 68 min. work, and finally a fall of 1·2 mg. p.c. In the second case on W. H. O. in which a small increase was found in the second work sample, but not in the first, the subject who was still quite comfortable after 55 min. had by the time of the second sample commenced to find the work irksome, and both subjects had the impression that the lactate rise found corresponded roughly to the feeling of discomfort due to the saddle. It would be interesting to compare similar degrees of exercise, using different saddles and cycle frames.

In all these experiments, again, the changes of CO_2-combining power found agree with those of lactate, and adequately control the latter.

The value of these results, as showing the persistence after cessation of exercise of small lactate changes, has already been mentioned.

Alveolar CO_2 *changes following light exercise.* The accumulation, and subsequent removal, of lactic acid in the blood and tissues are accompanied by definite changes in the alveolar CO_2 pressure of the individual, changes which—as mentioned in the Introduction—have been clearly demonstrated in the case of severe exercise. An attempt was therefore made to follow the alveolar CO_2 changes in the case of the types of exercise studied throughout the present research, the object being to provide a control of yet another type on the direct lactate analyses. The alterations which occur are small, and to provide any really adequate investigation would require a separate research. Looking at the graphs (p. 226), which give separately the results on W. H. O. walking and performing ergometer work, and on C. G. D. walking, it is evident that in every case there was a fall of the alveolar CO_2 pressure, soon after cessation of work, to a level below the resting value found in the same experiment. In the walk experiments performed on both subjects this fall seems greatest at a period 10 to 15 min. from the end of the walk, and if it were due to a lactic acid increase in the body there would seem no reason for the delay. It would appear rather more likely that the fall was due to some other factor, such as a temperature change in the body as a result of the exercise, though to be sure of any effect of this nature would need a much fuller investigation than possible in conjunction with the present research. In the three ergometer experiments on W. H. O. the fall seems to have reached its lowest point within the first few minutes from the cessation of exercise, and in contrast to the above

is just such as might be expected to follow an increase of lactic acid in the body.

In the walk experiments on both subjects in analogous conditions no lactate rise was found, so that the alveolar CO_2 results agree with this, but in the ergometer experiments on W. H. O. little or no lactate rise was found. The conditions in these two sets of experiments were, however, not really comparable, since those experiments performed for the investigation of alveolar air changes were carried through at an early stage of the research, when the subject had done no cycling for a year, and were performed on a Martin ergometer which was very uncomfortable for him, whereas the lactate experiments were done late in the research, after the subject had done a fair amount of cycling, and on the far more comfortable Krogh ergometer. It is quite possible, therefore, that a lactate increase did occur, such as would be expected from the alveolar changes found. With so few experiments, however, it is only permissible to say that the two sets are more in agreement than opposition.

Changes of inorganic phosphate in the blood following light exercise. The results given in Table X have already been discussed with regard to their bearing on changes in CO_2-combining power of the blood. The only systematic work published on inorganic phosphate changes in the blood during exercise is that of Havard and Reay(38), who found that immediately after short severe exercise there is a rise in concentration of the blood phosphate, followed at once by a fall to below the resting level. This fall was less in trained than in untrained individuals. The usual maximum for the rise was about 10 p.c. If the work were less severe, but longer continued (*e.g.* up to 15 min. running) the rise tended to be greater, reaching a maximum of about 25 p.c. No relation could be found between phosphate and lactate changes. In none of these experiments was the exercise really light (though Dr Havard has kindly told me that in two unpublished experiments no phosphate increase in the blood was found to follow walking exercise), and the present results are mainly of interest as supplying data for such light exercise. An increase was regularly found to follow the walking exercise, and was usually between 5 and 8 p.c. of the resting values obtained. The samples were taken at an average time of about 45 sec. after the end of the walk, but no attempt was made to obtain samples at later periods to see if any fall subsequently occurred.

Oxygen debts following light exercise. In the case of severe exercise it seems clear that the bulk of the oxygen debt must be incurred in the removal of excess lactic acid which has accumulated throughout the

tissues of the body. If we regard the absence of an increase in the lactic acid in the venous blood from inactive muscles during light exercise as evidence that no generalized increase in the lactic acid in inactive tissues has occurred, and probably no increase in active muscles, a different explanation of the oxygen debt must be found in such circumstances. No investigation of this point has been carried out, but for reference the oxygen debts were measured in the case of the exercise studied in the present work, and for both subjects came to about 1 l. of oxygen (Table XI).

VI. Conclusions.

For the purposes of making any comparison between the results obtained by different workers on a given subject a knowledge of the experimental conditions in each case is essential. These may therefore be now repeated for the present work. The experiments were performed throughout on the same two subjects, who differ markedly in age, build, etc. The subjects were in every case in the post-absorptive state, and the experiments were carried through as far as possible away from normal meal hours. The blood samples were taken from veins of the forearm, and thus represent venous blood from an inactive limb, in which a free circulation had been established, either by submerging the arm in hot water for a few minutes before taking the sample, or as a result of the general increase of blood flow following the exercise. Care was always taken to avoid any constriction of the blood vessels during the taking of the samples, and light pressure was applied to the arm during the introduction of the needle into the vein only.

Working on blood samples obtained in this manner it was found that there was no lactate increase as a result of exercise up to a certain critical level. This critical level varied in one individual for different types of exercise, and with training, and differed in different individuals. For both the present subjects, and for walking exercise, this critical level corresponded to an oxygen utilization of about 1·8 l. per min.

These results obtained from lactate analyses have been confirmed by a parallel study of changes in CO_2-combining power of the blood, and a few observations on alveolar air changes seem also to fall in line with what would be expected from the foregoing.

It is obvious that these results differ fundamentally from those obtained by Hill, Long and Lupton, and by Long (Table I of the Introduction), which showed a lactate increase in the blood even following

very slow walks. There seems no important difference between the conditions of the two sets of experiments, and no suggestion can be offered to account for the discrepancy. The various steps of the analytical procedure have been checked as far as possible, wherever it was considered that they could affect the results, but with no success in showing up any relevant factor. Thus, since the Clausen method gave higher values on blood than the Friedemann method used here, it was thought possible that it would also show some difference in the blood lactate as a result of exercise in cases where the present method did not do so, and controls were performed to test this. Two experiments of this type are given in Table XIII, and are clearly negative. Also we have the fact that bicarbonate analyses have controlled the lactate analyses in the present work. In view of these results it is possible that the explanation of the discrepancy between the two series of experiments is to be found in differences of response of individual subjects under apparently similar experimental conditions.

Accepting the results here obtained it is not easy to give a definite interpretation. In the first place it is possible that there was actually an increase in the lactate concentration of the venous blood passing to the general circulation from the active muscles, but that this had become masked before the blood reached the venous side of the circulation in the inactive arm muscles. If this were the case, since adequate time must have been allowed for the establishment in the experiments of approximate equilibrium, this removal of lactate could scarcely be a mere storage of lactate, but must have been an active process of oxidation and resynthesis. From this it would seem to follow at least that in the inactive tissues in light exercise the lactate concentration is maintained at resting level.

The writer tends to accept a second explanation, that in such light and normal exercise as walking there is no output of lactic acid from the active muscles so long as the oxygen supply can be maintained adequate to all the active tissues. This does not mean that the maximal oxygen uptake of the whole body be not exceeded, but rather that there exist no local areas of anoxæmia, a matter which will depend largely on the development and responsive activity of the capillaries and arterioles in the active muscles. It is quite possible that there might be a large blood flow to a tissue, but if the capillaries were widely spaced there could still exist between them areas of relative anoxæmia during activity of the tissue, and the critical level of exercise at which the lactate began to increase in the blood would depend upon the point at which this

anoxæmia commenced to make itself felt in the particular set of muscles used in the given exercise. Once the critical level were exceeded it would be necessary for the active fibres to hand on to others, more favourably situated, part of their lactic acid to be oxidized or resynthesized, but up to that time there would seem no *a priori* reason why any should be allowed to escape from the fibre in which it was produced.

The results of Hartree and Hill(18) on the relation between recovery rate and lactate concentration, obtained from a study of heat production in muscle tissue, do not disagree with these results, as they are concerned only with the conditions within the fibres.

On such a theory as that supported here the differences between the effects of different types of exercise are easily understood, since the more normal the exercise the more adequate is the circulatory system likely to be to supply oxygen to all the active tissues. Training will also have the effect of improving the local conditions, with the result that for the exercise in question the critical level is raised, as was shown most strikingly in the experiments on de Mar already discussed, which also seem much more readily explicable in the light of the present results than those of Hill, Long and Lupton.

VII. Summary.

1. Changes in the blood as a result of light exercise, especially walking, have been studied on two subjects. The subjects were always in the post-absorptive state at the time of the experiment, and the results apply in every case to venous blood from an inactive limb, through which there was a free circulation.

2. A critical metabolic level was found below which there was no increase in blood lactate as a result of the exercise, although above this level such an increase did occur.

3. The critical level varies for different subjects, and for the same subject for different types of exercise. For the present subjects and for walking exercise it corresponded to an oxygen consumption of about 1·8 l. per min.

4. There is a fall of CO_2-combining power of the blood above the same critical level, but not below it.

5. There was found in every case studied a slight increase in the inorganic phosphate of the blood, following exercise, even when no lactate change occurred.

6. A few observations have been made on alveolar CO_2 pressure changes in similar light exercise.

7. The oxygen debt in very light exercise has little relation to a lactic acid accumulation in the blood, and probably none to the concentration of lactic acid in the tissues at large in the body.

The writer wishes to acknowledge his indebtedness to Dr C. G. Douglas for his guidance and help throughout the research, and for having acted as subject in many of the experiments, and also to Prof. R. A. Peters for his help and for facilities granted.

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THE RESISTANCE OF THE PULMONARY VASCULAR BED.

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It is generally held that natural inspiration causes a diminution in the resistance of the pulmonary vascular bed. This opinion is, for the most part, founded upon experiments on the entire animal in which measurements of the systemic and arterial pressures are made after closure of the chest wall, and also upon the so-called "glass chamber" experiments in which the isolated perfused lungs are placed in a chamber for the purpose of negative pressure ventilation. References to most of the literature have been given in articles by Tigerstedt(1, 2) and Wiggers(3). There are, however, important experiments on record, not altogether in agreement with this view, which of late years have attracted considerable attention and make it desirable that the question should be reinvestigated.

Lewis(4), working on cats under natural respiration with vagi cut, was able to eliminate cardiac effects at will by exposing the heart to the atmospheric pressure. As a criterion of a diminished resistance in the pulmonary vascular bed, he used the appearance of the "velocity" curve in the systemic arterial pressure during sustained inspiration. He found in the smaller animals (2·0 to 2·5 kg.) the rise of systemic arterial pressure accompanying suspended inspiration to be entirely due to changes in intrapericardial pressure, and in no measure the result of a decreased resistance to flow through the pulmonary vessels. In larger cats such a conclusion was not warranted, although in these animals the increased diastolic filling of the heart played a large part in causing the inspiratory rise in systemic pressure. No definite opinion was expressed with regard to the changes occurring in dogs.

Cloetta(5, 6, 7), from observations on a lung lobe placed in a plethysmograph under reduced pressure, concluded that shallow inspirations in the dog lower the resistance, and deep inspirations increase the resistance especially at the maximum height of the inspiration. Visscher, Scott and Rupp(8) have returned to the earlier view held by Quincke and

Pfeiffer[30], on the grounds that they find that the extent to which the lungs and lung capillaries are expanded is the same for a given pressure difference between the intrathoracic and intrapulmonary spaces, irrespective of whether positive or negative pressure is used for expanding the lungs. From these results they argue that natural inspiration and positive pressure inflation have the same effect on the lung blood vessels. Their conception of the events taking place in the whole animal is that, although pulmonary resistance is raised during inspiration, the blood flow through the lungs is augmented in consequence of the increased diastolic filling of the heart. Cloetta is in disagreement here, for in order to bring the lungs to the same volume, he finds a greater intratracheal positive than intrathoracic negative pressure is necessary. More recent work by Chillingworth and Hopkins[9, 10, 11] on the dog placed in a reduced pressure chamber, but with the intrapulmonary space in connection with the outside air, inclines them to the belief that the pulmonary resistance is raised with deep inspiration. An analysis of the literature on the subject made it apparent that, before proceeding to a new mode of attack of the problem, there were certain aspects of the work by the earlier investigators upon which it was desirable to throw further light. In de Jager's[12] "glass chamber" experiments the blood flow rarely exceeded 100 c.c. per minute, a value much below the normal, and it is possible that the resistance alterations in response to lung expansion are determined in part by the prevailing blood flow, and by the initial distension of the pulmonary blood vessels. A number of experiments have been carried out on the isolated perfused lungs under negative pressure ventilation, using large blood flows in order to test this possibility. Cloetta's contention that great expansion of the lungs increases the vascular resistance has also been tested. It was apparent that the findings of Lewis were of outstanding importance, since the method he employed produced a smaller disturbance of the natural physiological conditions than those which hitherto had been used by other workers. Lewis's experiments, therefore, were repeated and extended in the hope that additional information might be obtained. A further study of the problem has been made with the closed circuit heart-lung preparation under negative pressure ventilation, the preparation being arranged to allow of the heart and the lungs, separately or together, to be brought under the influence of the reduced chamber pressure. By this means the cardiac effects which usually accompany lung expansion are able to be eliminated.

EXPERIMENTS ON THE ISOLATED PERFUSED LUNGS OF THE DOG.

Method.

The lungs are set up in the negative pressure chamber and perfused with defibrinated blood in the manner previously described(13). Two blood velocity recorders of special design(14) are inserted in the circulation in order to measure the rate of flow of blood entering and leaving the lungs. The flow recorders are of the Chauveau-Lortet type (Fig. 1, A)

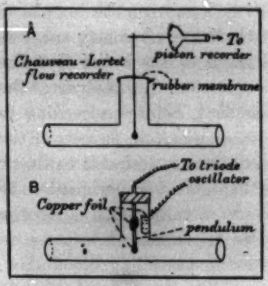


Fig. 1.

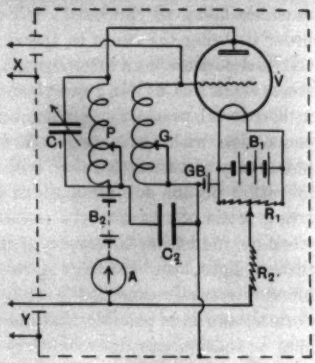


Fig. 2.

Fig. 1. See text.

Fig. 2. V=D.E.P. or D.E. 5 triode. G=grid inductance of 75 turns on 2 inch former, tapped. P=anode inductance similar to G. B_1=low tension batteries. B_2=high tension batteries, 120 volts. C_1=condenser 0·05 $\mu\mu$F., variable with reduction gear control. C_2=condenser 2·0 μF. R_1, R_2=300 ohms. The leads at X are connected to the flow recorder, those at Y to the galvanometer. GB=grid battery.

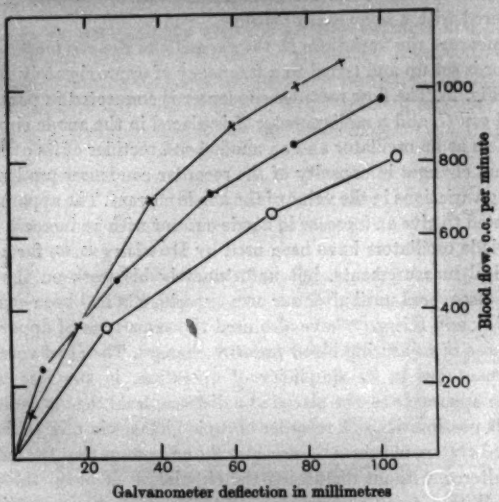
and are capable of registering quick changes in flow occurring during the respiratory cycle. A slight modification in the recorder is made to exclude the error due to the stretching of the rubber membrane which is found to occur as a result of pressure changes if the usual type is used. The pendulum (Fig. 1, B) consists of a watch spring on to which two pieces of copper foil are soldered; the spring is fixed in the central arm of a T-tube so that the lower foil lies in the blood stream. The upper foil is attached to the middle portion of the watch spring, and alters its position in relation to the side of the central arm when the velocity of blood changes. To the outside of the central portion of the T-tube is

fixed another piece of foil, and this, in conjunction with the upper foil attached to the pendulum, form the two plates of an electrical condenser, the capacity of which varies with the rate of blood flow. The foils are covered with a light coat of shellac.

To measure the variations in the capacity of the condenser, a triode oscillator is set up and tuned to a frequency of approximately 1000 kilocycles (Fig. 2). The flow recorder condenser is connected in parallel with the grid coil *G*, and a milliammeter *A* is placed in the anode circuit. The triode acts as an oscillator and an anode bend rectifier of its own oscillations, and changes in capacity of the recorder condenser produce corresponding variations in the value of the anode current. The apparatus may be adjusted to give an increase in anode current with an increase in blood flow. Triode oscillators have been used by Dowling(15, 16) for a variety of physical measurements, but unfortunately his work on the subject was not discovered until after our own experiments had been completed. Braddick and King(17) have also used the same type of apparatus for the purpose of measuring blood-pressure changes. The chief value of the arrangement lies in its simplicity of operation, in the fact that the recording apparatus can be placed at a distance from the operating table, and in its possibilities as a recorder of rapid changes in flow taking place in a closed system of tubes; there is also the advantage that the sensitivity can be altered without disturbing the circulation of blood through the T-tube. With a small power receiving triode, used in conjunction with a Salmonson galvanometer as an anode current measurer, changes in flow of 5 c.c. per minute can be detected. The sensitivity of the oscillator is most conveniently altered by varying the capacity of the anode or grid circuit. Fig. 3 shows three curves obtained in this way.

Dowling has given a mathematical analysis of the conditions governing the performance of such an oscillator, and of the various methods of adjustment, but there are a few additional points which are worthy of mention if it is desired to use the oscillator as a flow recorder. Experience has shown that the relation between blood flow and anode current change does not remain constant during long experiments. The anode current "creeps" to a small extent, even if the usual precautions are taken of using large capacity high tension batteries, and of allowing the triode to heat up before the records are taken. This effect is, at any rate, in part due to the high temperature which is reached by the triode when set up as an oscillator, and might be overcome by using one designed for the purpose. An elaborate earthing system must be employed, as movements of the animal such as occur during the re-

spiratory cycle are apt to influence the records. If quantitative measurements are required, it is necessary to calibrate the instrument before and



Weston relay: optical magnification 13. Triode = D.E.P. 215. H.T. = 60 volts.

Fig. 3. See text.

after each experiment under actual working conditions; for this purpose a Stolnicov stromuhr is connected in series with the Chauveau-Lortet recorder and the mean blood flow obtained.

The variations in anode current have been taken with a Salmonson string galvanometer or a Weston P.O. relay placed in the anode circuit, the constant anode current being balanced out in the conventional manner.

The pulmonary arterial and the respiratory (intrathoracic) pressures within the glass chamber are measured with membrane manometers, and the volume of the external circuit with a piston recorder. The movements of the various levers are recorded by means of a Cambridge photographic plate camera. A test showed that no appreciable lag existed in the recording systems, so that a vertical line on the tracings passes through corresponding ordinates. A typical record is illustrated in Fig. 4.

Results.

In de Jager's[18] writings will be found the statement that, with a constant perfusion pressure, the flow through the lungs expanded by a

Fig. 4. Isolated perfused lungs under negative pressure ventilation. *R.P.* = respiratory pressure, inspiration downwards. *B.V.* = blood volume of external circuit, a downward movement of the lever denotes an increase in blood volume of the lungs. *B.F.* = blood flow leaving lungs, a movement upwards denotes an increase. *P.A.* = pulmonary arterial pressure. Respiration = 14 per minute. Mean blood flow = 350 c.c. per minute. Mean perfusion pressure = 20 mm. Hg.

negative pressure is greater than that through the collapsed lungs. In our experiments the lungs are perfused by a pump, the output of which remains constant; a fall in the resistance of the pulmonary vascular bed will be shown, therefore, by a diminution in the pulmonary pressure. In a large number of experiments, it has been found that the pulmonary pressure is lower when the lungs are expanded than when collapsed, the blood flow remaining unaltered, or even being slightly increased (Figs. 5, 6). In Figs. 4 and 5 a slight increase in the blood flow entering the lungs is shown when the lungs are expanded and the pulmonary pressure is lower; there can be no doubt, therefore, of the smaller resistance of the expanded lung when compared with the collapsed. The slight increase in flow is no doubt due to the fact that less of the pump energy is utilized in distending the rubber tubing and the arteries at the lower arterial pressure, since the frequency and the stroke of the pump remained constant. The blood volume curve indicates a larger quantity of blood in the expanded than in the collapsed lungs.

De Jager's results, therefore, are confirmed for larger blood flows

than he employed; in this series of experiments the maximum blood flow amounted to 700 c.c. per minute.

Fig. 5. Dog, 5·0 kg. Isolated perfused lungs; negative pressure ventilation. Mean blood flow 500 c.c. per minute. Mean perfusion pressure 30 mm. Hg. Upper tracing, intrathoracic pressure; second, blood volume of the lungs, increase shown by a downward movement of the lever; third, arterial blood flow; fourth, pulmonary arterial pressure. A complete respiratory cycle followed by a suspended expiration giving way to a suspended inspiration is shown. During suspended inspiration the flow is slightly larger, the pulmonary arterial pressure smaller and the blood volume of the lungs greater than during suspended expiration. Lettering as in Fig. 4.

Fig. 6. Same experiment as Fig. 5. A sustained expiration follows a sustained inspiration.

No evidence has been found of Cloetta's contention that excessive expansion of the isolated perfused lungs increases the resistance of the

vascular bed. A respiratory pressure of − 15 mm. Hg is usually more than sufficient to expand fully the lungs of a 10 kg. dog, yet pressures of − 20 mm. Hg in a number of experiments and of − 28 mm. Hg in one experiment caused a drop in the pulmonary pressure, the flow remaining constant.

Experiments on the heart-lung preparation.

Experiments on the whole animal by Mollgaard(19) have shown that a sustained negative pressure surrounding the heart and lungs causes a large augmentation in pulmonary flow, a result which has been confirmed on the heart-lung preparation(20). With the aid of Stewart's(21) method for recording the axial velocity of the blood stream, Romm(22) noted an increase in pulmonary flow during deep inspiration, although Langlois and Dubois(23) were unable to confirm this result. In these experiments there is no indication as to whether the flow augmentation is due wholly to the increased diastolic filling of the heart, or whether the lungs play some part.

In the experiments to be described, dogs have been used fully anæsthetized with chloralose, 0·1 g. per kg. body weight being injected intravenously. The arrangement for allowing the respiratory pressure to exert its effect on the heart and on the lungs separately or together is seen in Fig. 7. The apparatus used(20) for the respiratory pressure variations for ventilation of the lungs has been previously described, the variations are applied through the lead pipe marked *a*, *b*, *c*, the same variations being transmitted to the heart through a second pipe *a*, *d*, *e*, which connects with the interior of the cardiometer. Side tubes able to be opened or closed at will are inserted in the channel portions *b*, *c*, and *d*, *e*, at 1 and 4 respectively. These channels can also be cut off from communication with the respiratory pump at the points marked 2 and 3. For the sake of clearness 1, 2, 3 and 4 are shown as taps, in practice, artery forceps are used to clamp stout rubber tubing inserted in the lead pipes at these points. When the pressure variations are applied to the heart and lungs, 1 and 4 are closed, 2 and 3 are opened; when applied to the lungs alone, 1 and 3 are closed, 2 and 4 are opened, the heart then being at atmospheric pressure. With this arrangement it is also possible to keep the heart and lungs at different negative pressures. The cardiometer encloses the whole heart and is pushed as far as possible towards the base, and is then tied in position with a stout ligature encircling the pericardium.

A certain amount of difficulty was experienced in obtaining reliable

comparisons of blood flow in relation to the value of the negative pressure exerted upon the lungs; this was due to the steady diminution

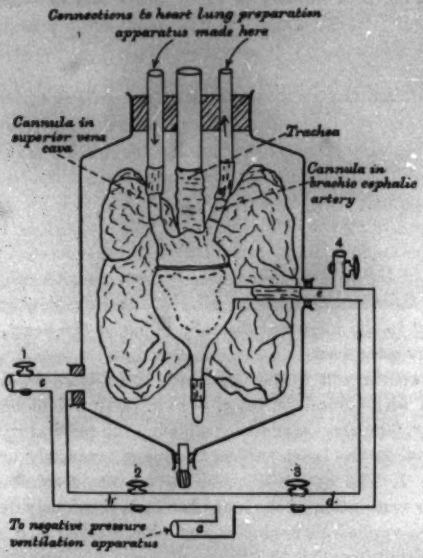


Fig. 7. See text.

in flow caused by blood leak from the preparation. It was overcome by taking a series of observations over long periods and plotting the results on squared paper. Fig. 8 shows such a series of the effect upon the peripheral blood flow of reducing the respiratory chamber mean pressure, and then bringing it back to approximately its initial value. In Fig. 8, *A* and *C*, the reductions of respiratory pressure, affected both the heart and the lungs; in *B* and *D*, the lungs only were influenced by the respiratory pressure, the heart being at atmospheric pressure. It will be seen that there is a definite increase in peripheral flow when the lungs are expanded by a negative respiratory pressure, the outer surface of the heart being at atmospheric pressure. Two other experiments gave similar results. Further evidence that lung expansion alone augments the flow is afforded by another type of experiment (Table I), in which it is found that the peripheral flow is larger when the mean respiratory

TABLE I. Closed circuit heart-lung preparation. Influence of the respiratory pressure upon the heart alone, upon the lungs alone and upon the heart and lungs together.

Exp.	Dog (wt. kg.)	M.B.P. (mm. Hg)	P.O. (c.c. per min.)	R.P. (mm. Hg) Insp.	Exp.	Mean	R.P. effective upon
1 *a*	7·0	87	245	−5	−2	−3·5	H. + L.
		84	190	−5	−2	−3·5	L. only
		88	245	−5	−2	−3·5	H. + L.
		83	180	−4	−1	−2·5	L. only
1 *b*	—	74	355	−8·5	−6	−7·25	H. + L.
		70	235	−7	−6·5	−6·75	H. only
		73	355	−8	−6	−7·5	H. + L.
		70	235	−9	−6	−7·5	L. only
		74	320	−8	−5	−6	H. + L.

M.B.P. = mean blood-pressure. P.O. = peripheral output. R.P. = respiratory pressure. H. = heart. L. = lungs.

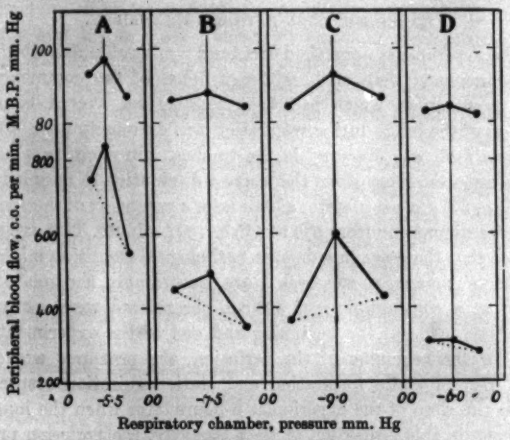


Fig. 8. *A* and *C*, effect of a negative pressure on the heart and lungs. *B* and *D*, effect of a negative pressure on the lungs alone. See text.

pressure influences the whole preparation than when it acts either on the heart alone or on the lungs alone; therefore, both cardiac and lung effects are responsible for the increased heart output. At the moment it would appear that the only feasible explanation of this flow augmentation due to lung expansion is a reduction in the total resistance of the vascular bed. The possibility that the negative pressure, when effective upon the lungs alone, indirectly influences the venous filling of the heart by dilating the portions of the auricles situated in the deeper part

of the pericardial sac cannot be definitely excluded, but is unlikely since the cardiometer was pushed well upwards, and the pericardium stretched tightly over the lip of the cardiometer and there fixed in position.

It is true that the peripheral and not the pulmonary flow has been measured in these experiments, but it is reasonable to assume that if the peripheral flow increases with a concomitant rise in peripheral blood-pressure, the coronary flow will also increase, and this will mean an augmented pulmonary flow. A local cardiopulmonary reflex, causing constriction of the coronary vessels in response to lung expansion, is a possible but unlikely explanation of the results. In this event, instead of an increased pulmonary flow being responsible for the raised peripheral flow, the latter would take place merely by the effect of the coronary constriction shunting more blood round the peripheral circulation.

Experiments on pathological lungs.

In the experiments upon the heart-lung preparation described above the measurements were made within 2 hours of the commencement. In one preparation, which had been running for over 4 hours, the expansion of the lungs during respiration was extremely poor, although no œdema fluid was observed in the trachea. On exerting a negative pressure upon the lungs alone there was a diminution in peripheral flow from 375 to 275 c.c. per minute; a little later a repetition of the procedure produced a diminution from 430 to 415 c.c. per minute. This experiment suggested that the lungs in a definite pathological condition might react in a different manner to the fresh lungs. Accordingly, the isolated lungs perfused at a constant flow of 300 c.c. per minute were set up, and observations made at the beginning and end of the experiment. One hour after the beginning of the perfusion, the pressures were 7 and 19 mm. Hg for static inspiration and static expiration respectively. Towards the close of the experiment 6 hours later when the lungs had lost a considerable proportion of their elasticity, the perfusion pressures were 14 and 12 mm. Hg for static inspiration and static expiration respectively. This indicates that the pathological lungs (perfused) do not diminish their vascular resistance on expansion; a second experiment gave the same result. In this connection, Jarisch and van Wijngaarden(24) found that the perfusion pressure first decreased with the continuation of perfusion, but finally increased with the onset of œdema. This finding has been confirmed and, in addition, it has been observed that a preliminary inflation of the lungs at the commencement of the experiment reduces the perfusion pressure considerably, and as the experiment progresses the pressure falls but little; this procedure has been adopted in the large

majority of experiments and has the advantage of producing stable conditions in the lungs for at least 2 hours.

Experiments on the entire animal.

As previously stated, Lewis's method for obtaining suspended inspirations with the heart exposed to atmospheric pressure has been used. The procedure described as Method III in his paper has been followed in detail with the exception that 0·43 g. of medinal (Schering-Kahlbaum) per kg. body weight is slowly injected intravenously after induction with ether; Lewis anæsthetized with urethane and A.C.E. mixture. In our experiments the cannula tied into the pericardium had a bore of 6 mm. and its position was carefully observed post-mortem. Five experiments on cats were performed. In three cats (3·1, 1·8 and 2·0 kg.) the "velocity" curve in the systemic arterial pressure tracing during suspended inspiration disappeared completely on exposing the heart to atmospheric pressure. In one cat (2·5 kg.) the "velocity" curve with the heart at atmospheric pressure was present, but varied in size without any relation to the depth of respiration. In one cat (1·6 kg.) typical vagal breathing failed to appear, but prolonged inspirations occurred singly or in groups of two or three following respirations of normal frequency. During the prolonged inspirations the "velocity" curve was well marked when the heart was exposed to atmospheric pressure (Fig. 9). In these two animals which showed the presence of the "velocity" curve when cardiac effects were eliminated it was less than when the pericardium was closed. The results confirm those of Lewis in that the "velocity" curve fails to appear in some animals during prolonged inspiration when the heart is exposed to atmospheric pressure. It is possible that vasomotor changes in the peripheral circulation occurring synchronously with respiration might account for the non-appearance of the "velocity" curve in some of the experiments, for Fredericq[25] has shown that in the vagotomized dog under artificial respiration with the thoracic and abdominal cavities laid open and phrenic nerves cut, a cessation of the artificial respiration leads to respiratory efforts on the part of the animal, during which the systemic arterial pressure shows an inspiratory fall and an expiratory rise. Fredericq's experiments have been repeated on the cat and his findings confirmed. There is a small but definite fall in systemic arterial pressure during inspiration (Fig. 10); this being so, the non-appearance of the "velocity" curve during sustained inspiration in cats with the pericardium open to the outside air, might be explained by a fall in

systemic resistance neutralizing the effect upon the blood-pressure of a fall in the resistance of the pulmonary vascular bed. In Fig. 10 the

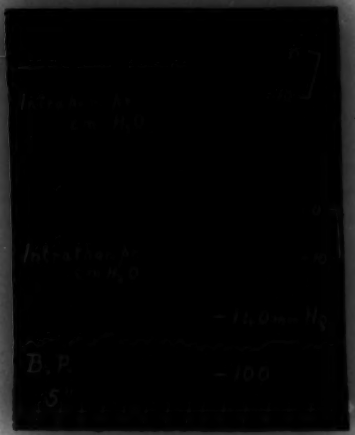


Fig. 9.

Fig. 10.

Fig. 9. Cat; vagi cut, 1·6 kg. Appearance of the "velocity" curve with the heart exposed to atmospheric pressure. See text. Top tracing, intrapericardial pressure; second, intrathoracic pressure; third, carotid pressure; fourth, 5-sec. intervals.

Fig. 10. Cat, 2·0 kg., vagi and phrenic nerves cut, thoracic and abdominal cavities widely opened. Animal making respiratory efforts during light artificial respiration; at arrow the artificial respiration is stopped. The succeeding respiratory efforts are accompanied by variations in the blood-pressure. *Resp.* = movements of the thoracic wall, inspiration upwards. *B.P.* = carotid blood-pressure.

positions of the ordinates appear to show that the blood-pressure commenced to fall before the onset of inspiration, but inspection of the chest walls revealed a slight contraction of the intercostal muscles occurring before the lever recorded a movement. Unfortunately the cats in this series of experiments did not exhibit prolonged inspiratory efforts and, therefore, it is not possible to say definitely that peripheral dilatation is responsible for the non-appearance of the "velocity" curve in some of Lewis's and of our own experiments. I think it is clear that if, under certain conditions, the resistance of the systemic circulation undergoes respiratory variations, it is unsafe to assume that systemic arterial pressure changes are a criterion of alterations taking place in the

resistance and the flow through the pulmonary vascular bed. Since peripheral circulatory disturbances have not been excluded in these experiments, the non-appearance of the "velocity" curve does not definitely militate against prolonged inspiration causing a fall in the resistance of the lung blood vessels.

Discussion and conclusions.

The results of the investigation as a whole reveal no evidence of Cloetta's contention that expansion of the lungs gives rise to an increased resistance in the lung blood vessels; on the contrary, the experiments on the perfused isolated lungs and on the heart-lung preparation point to there being a definite decrease. Cloetta bases his opinion upon a comparison of the microscopic appearances of lung tissue fixed in the collapsed and expanded (by negative pressure) condition, and also upon the observation that the pulsations at each heart beat of a lung lobe placed in a plethysmograph are smaller when the lobe is expanded than when collapsed. He considers diminished pulsations of blood vessels to indicate an increase in their resistance, but Bruns(26) has criticized this interpretation, because diminished pulsations of the elastic vessel walls will occur as a natural sequence of an increased flow; this criticism is undoubtedly a valid one. Moreover, Heinbecker(27) is unable to accept the histological evidence which Cloetta brings forward in support of his views.

The investigations on the entire animal in which the presence of a "velocity" curve in the systemic arterial pressure is taken as a criterion of a diminished resistance in the lung blood vessels after cardiac effects have been eliminated, give results which are not so conclusive. It does seem, however, that, in view of the possibility of an inspiratory fall in the systemic resistance, the failure of the "velocity" curve to appear during prolonged inspiration in some cats cannot be considered as an unequivocal argument against the acceptance of the theory of lung expansion giving rise to a diminished pulmonary resistance in these animals. Moreover, a diminished pulmonary resistance does not of necessity mean an increased pulmonary flow, for unless the flow to the right heart is adequate and the right heart is in a condition to respond, by increased output, to the diminished pulmonary resistance, no velocity curve will appear. Before any final conclusion can be reached on these lines, a further investigation is clearly indicated.

It may be argued with truth that the experimental conditions of the heart-lung preparation are far from normal, but the problem demands

careful control of the blood supply to the lungs and the elimination of blood capacity effects and of nervous influences which lead to errors in interpretation of the results, controls which are not obtainable in the entire animal. For this reason the value of the investigation lies chiefly in the addition which has been made to the already vast weight of evidence, obtained with a variety of methods, in favour of lung expansion being accompanied by a fall in pulmonary vascular resistance. The "glass chamber" experiments[1] with the isolated perfused lungs (de Jager(12)), the experiments on the perfused lungs with diaphragmatic respiration and intact thorax (d'Arsonval(28)), and the analysis of optical records of pulmonary arterial pressures in the intact animal (Wiggers(29)) all support this view.

Summary.

1. In the isolated lungs (dog) perfused with defibrinated blood by a constant output pump and ventilated by negative pressure, a reduction in the mean respiratory pressure (intrathoracic) leads to a fall in the resistance of the pulmonary vascular bed. After prolonged perfusion when the lungs have lost most of their elasticity, expansion fails to lower the resistance of the vascular bed.

2. In the heart-lung preparation (dog), with the heart exposed to atmospheric pressure and the lungs ventilated by a negative pressure, a reduction in the mean respiratory pressure increases the output of the heart. Reasons are given for considering this as being due to a fall in the resistance of the lung blood vessels.

3. The absence, during prolonged inspiration, of the "velocity" curve in the systemic arterial pressure of vagotomized cats with the heart exposed to atmospheric pressure and the chest walls intact, does not of necessity militate against a concomitant fall in the resistance of the lung blood vessels.

[1] In the "glass chamber" experiments, if during static inspiration the perfusion pressure is reduced by the same amount as the chamber pressure, the blood flow is diminished (Quincke and Pfeiffer(30), Funke and Latschenberger(31), Bowditch and Garland(32)), but if the perfusion pressure remains constant the blood flow increases (de Jager(12, 13, 33, 34, 35)).

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SINUS CAROTICUS AND RESPIRATORY REFLEXES.

I. Cerebral blood flow and respiration. Adrenaline apnœa[1].

By C. HEYMANS and JEAN J. BOUCKAERT.

(*From the Department of Pharmacology, University of Ghent.*)

If the isolated head of a dog *B* is perfused from the vessels of a dog *A*, and the only connections between the head of *B* and the isolated heart-lung preparation of *B* are the vagus-depressor nerves, one observes (1) that an increase or a decrease of the arterial blood-pressure in the cardio-aortic vascular area of *B* produces respectively a reflex inhibition, a reflex apnœa, or a reflex stimulation, a reflex hyperpnœa, of the respiratory centre of head *B*. The vagus-depressor nerves are the centripetal paths of these respiratory reflexes, in relation with the cardio-aortic blood-pressure.

It is known that the occlusion of the common carotid arteries stimulates and that an increase of blood-pressure, or blood flow, in the carotid cephalic circulation inhibits the activity of the respiratory centre (Magendies and Cooper(2), Kussmaul and Tenner(3), Rosenthal(4), Hill(5), Lumsden(6), J. F. and C. Heymans(1), Gesell(7), C. F. Schmidt(8)). These facts are taken by several authors as evidence that "the respiratory centre is influenced directly by changes in the blood supply...respiration is depressed by an increase in cerebral blood flow, stimulated by a decrease no matter how produced" (C. F. Schmidt). However, as early as 1900, Siciliano(9) pointed out that the respiratory reactions are more marked after the occlusion of the carotids than after occlusion of the vertebral arteries, although the latter are more important for the blood supply of the circle of Willis, and Siciliano concludes in favour of respiratory reflexes of carotid origin. In 1912, Sollmann and Brown(10) demonstrated that traction on the cephalic end of the common carotid produced respiratory reflexes. More recent investigations have shown that electric stimulation of the bifurcation of the common carotid (sinus caroticus) and the increase of

[1] A preliminary account of these experiments was read before the Physiological Society in London on January 18th, 1930.

pressure in the sinus caroticus produce respectively a reflex stimulation and a reflex depression of the respiratory centre (H. E. Hering(11), Danielopolu and his collaborators(12), Moissejeff(13), C. Heymans(14), and E. Koch(15)).

In order to obtain more definite information on the relations between blood-pressure, cephalic blood flow, and respiratory activity, we have performed the following experiments.

In dogs (Fig. 1) anæsthetized with chloralosane or urethane, the two common carotid arteries are cut between ligatures. The thyroid arteries and the efferent branches of the sinus caroticus (external carotids, internal carotids, occipital, laryngeal and lingual arteries) are tied, care being taken not to sever the sinus innervation. The cephalic ends of the common carotids are connected with the Dale-Schuster(16) pump; outflow cannulas are placed in the sinus efferent branches of the lingual arteries. By means of this technique, which is an adaptation of the method of perfusion of the isolated sinus caroticus which one of us described before(14, 17), the two isolated sinus caroticus (bifurcation of the common carotid and ganglion caroticum), connected with the animal only by the sinus innervation, are perfused under pulsating arterial pressure with defibrinated blood or Ringer solution of which one can modify the pressure, the flow, the pulsating rate and the physico-chemical properties. The dog's respiratory rate and volume, the heart frequency and femoral blood-pressure are registered.

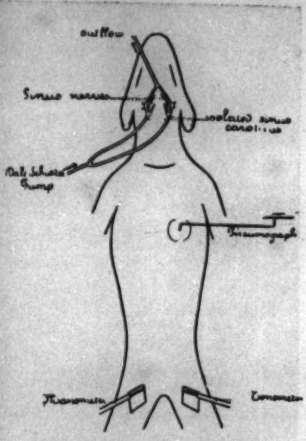


Fig. 1. Showing the method of perfusion of the two isolated sinus caroticus.

The two sinus caroticus being perfused at a low pressure of 60 mm. Hg (*S.P.* Fig. 2), the respiratory rate of the vagotomized dog is 28 movements per minute (*R.* Fig. 2). At ↑ *a*, Fig. 2, a rise of the sinus perfusion pressure to 300 mm. Hg produces an immediate cessation of respiration, a reflex apnœa, with a diminution of the general and cerebral arterial (vertebral arteries) blood-pressure from 260 to 120 mm. Hg.

This apnœa remains till the moment the sinus perfusion pressure is

lowered (↑ *b*, Fig. 2) to 150 mm. Hg; the respiration comes back immediately, and this before the rise of general blood-pressure.

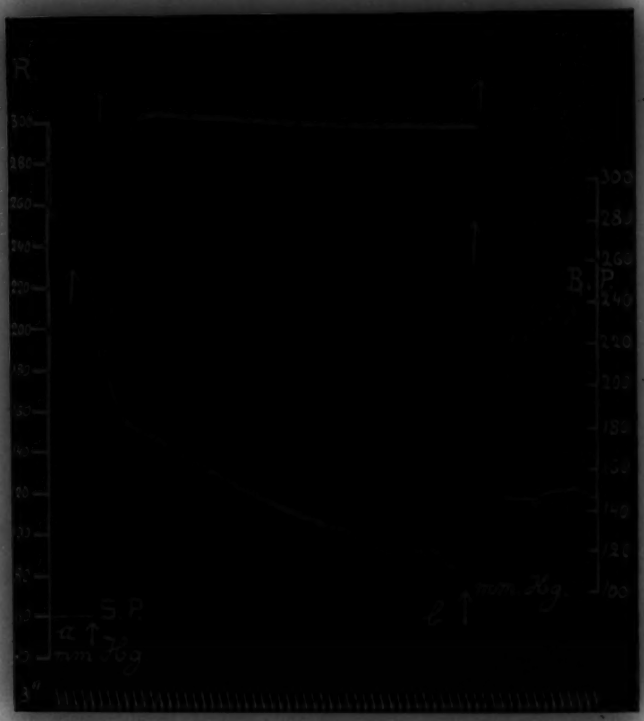


Fig. 2. Dog. 23·5 kg., chloralosane, vagi-depressor nerves cut. Perfusion of the two isolated sinus caroticus. *R.*, pneumogram. *B.P.*, femoral arterial blood-pressure, mercury manometer. *S.P.*, perfusion pressure of the sinus caroticus, mercury manometer. ↑ *a*, increase of sinus perfusion pressure. Apnœa and decrease of general blood-pressure. ↑ *b*, decrease of sinus perfusion pressure. Respiration returns and increase of general blood-pressure.

The two sinus caroticus being perfused at a pressure of 48 mm. Hg (*S.P.* Fig. 3) the respiratory rate is 16 movements per minute (*R.* Fig. 3).

At † *a* and † *c*, Fig. 3, the sinus pressure is increased to 220 mm. Hg; the respiration becomes immediately slower (*R.* Fig. 3) although the general and cerebral vertebral pressure decreases from 160 to 120 mm. Hg (*B.P.* Fig. 3).

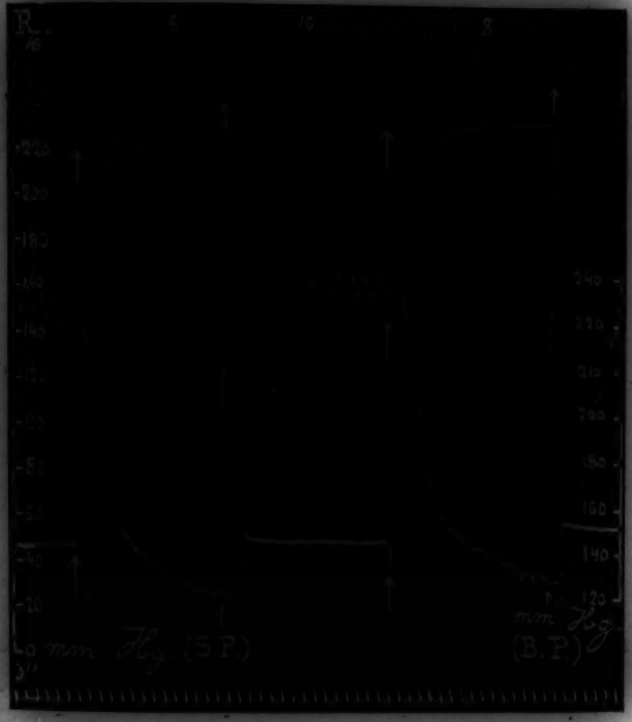


Fig. 3. Dog. 17·5 kg., chloralosane, vagi-depressor nerves cut. Perfusion of the two isolated sinus caroticus. *R.*, pneumogram. *B.P.*, femoral arterial blood-pressure, mercury manometer. *S.P.*, perfusion pressure of the sinus caroticus, mercury manometer. † *a* and † *c*, increase of sinus perfusion pressure. Reflex inhibition of the respiratory centre and decrease of general blood-pressure. † *b* and † *d*, decrease of sinus perfusion pressure. Reflex stimulation of the respiratory centre and increase of general blood-pressure.

At ↑ *b* and ↑ *d*, Fig. 3, the sinus perfusion pressure is lowered to 50 mm. Hg and the respiration of the animal becomes immediately faster (16 movements per minute). The general blood-pressure rises to 230 mm. Hg (*B.P.* Fig. 3). The respiratory reflex stimulation precedes the reflex changes of the general blood-pressure.

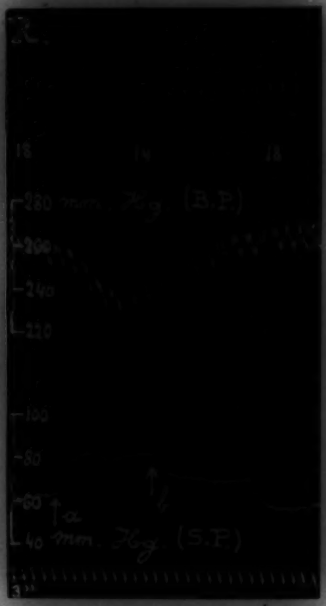


Fig. 4. Dog. 17·5 kg., chloralosane, vagi-depressor nerves cut. Perfusion of the two isolated sinus caroticus. *R.*, pneumogram. *B.P.*, femoral arterial blood-pressure, mercury manometer. *S.P.*, perfusion pressure of the sinus caroticus, mercury manometer. ↑ *a*, increase of sinus perfusion pressure from 60 to 80 mm. Hg. Reflex inhibition of the respiratory centre, 18 to 14 respiratory movements per minute. ↑ *b*, decrease of sinus perfusion pressure from 80 to 60 mm. Hg. Reflex stimulation of the respiratory centre, 14 to 18 respiratory movements per minute.

With the perfusion pressure (*S.P.* Fig. 4) of the isolated sinus caroticus at the level of 60 mm. Hg, one observes that a slight increase of sinus pressure to 80 mm. Hg produces a reflex slowing of the respiration

(*R.* Fig. 4), whereas a slight decrease of the sinus pressure causes an acceleration of the respiratory movements.

These experiments show that changes of arterial blood-pressure affecting only the isolated sinus caroticus reproduce the modifications of the activity of the respiratory centre which may be obtained by changing the blood-pressure in the cephalic circulation.

The reflex stimulation of the respiratory centre, by a low blood-pressure in the isolated sinus caroticus, also occurs in a hyperventilated dog; the humoral apnœa is neutralized by the reflex hyperpnœa. In the same way we observed that a central humoral hyperpnœa may be transformed into an apnœa by raising the blood-pressure in the sinus caroticus only.

In a previous paper(1) we have also demonstrated that a low blood-pressure in the cardio-aortic circulation only, may transform, by a reflex, the central apnœa into an hyperpnœa. These observations may perhaps explain the facts described by Fraser, Ross and Dreyer(18), Bald(19), Hertzmann and Gesell(20) of hyperventilation produced by hæmorrhage (low blood-pressure) although the blood is more alkaline.

Before the discovery of the important and predominant part taken by the sinus caroticus in the reflex regulation of heart rate and vasomotor tonus, it was generally accepted that cerebral blood-pressure acted directly upon the centres regulating the heart rate and the vasomotor tonus. The experiments of H. E. Hering and his collaborators (Koch, Kisch, Mies and Nordmann), Florey, Marvin and Drury(21), and our own observations(14) have demonstrated that the changes of cephalic blood-pressure and blood flow determine modifications of heart rate and vasomotor tonus only by a reflex set up by the blood-pressure changes acting on the sensitivity of the sinus caroticus. Acute anæmia or asphyxia has a direct central action, but if a lowering of cephalic arterial pressure produces a reflex (sinus caroticus) acceleration of the heart, acute anæmia on the contrary produces a central heart slowing. The question arises: are the immediate modifications of respiration produced by the changes in cephalic blood-pressure or blood flow also only of reflex, sinus caroticus, origin or are they partly of direct central origin? The following experiments were performed in order to answer this question.

In dogs, anæsthetized with chloralosane or urethane, all the efferent branches of the two sinus caroticus, except the lingual arteries, are ligatured, the depressor nerves are cut (method of E. Koch), threads

are placed, but not tied, under the vertebral arteries. The pneumogram and the arterial femoral blood-pressure are registered.

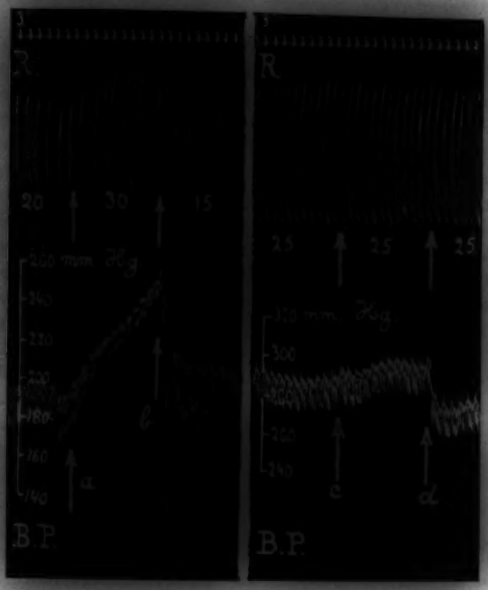
The clamping (↑ *a*, Fig. 5) of the two common carotids brings about

Fig. 5. Dog. 11·9 kg., urethane, depressor nerves cut. The efferent arteries of the two sinus caroticus, except the lingual arteries, are tied. *R.*, pneumogram. *B.P.*, femoral arterial blood-pressure, mercury manometer. ↑*a*, common carotid arteries are clamped. Reflex stimulation of the respiratory centre (24 to 40 respiratory movements per minute) and increase of the general blood-pressure. ↑*b*, common carotid arteries are opened. Reflex inhibition of the respiratory centre and decrease of general blood-pressure. ↑*c* to ↑*d*, vertebral arteries clamped, no changes in respiratory activity and blood-pressure.

a diminution of blood-pressure in the sinus caroticus only, the general blood-pressure of the dog rises from 165 mm. Hg to 220 mm. Hg; the respiratory centre is stimulated, the respiratory rate changing from 24 to 40 movements per minute. When the clamps on the common carotids are removed the respiratory rate slows down immediately to the previous rate of 24 movements per minute. The occlusion (↑ *c* to ↑ *d*, Fig. 5) of the two vertebral arteries does not produce any change in the activity of the respiratory centre.

In other experiments the efferent arteries of the sinus caroticus were not ligatured. The occlusion of the common carotid arteries (↑ *a*, Fig. 6, A) produces the normal acceleration of respiration (*R.*, Fig. 6) and the respiration slows down immediately when the carotids are opened again (↑ *b*, Fig. 6, A). The two sinus caroticus are then denervated between A and B, Fig. 6, and one observes that, although the general blood-pressure and cerebral blood flow are increased, the respiratory centre is not depressed but stimulated, and that the occlusion and

opening of the common carotids († *c*, † *d*, Fig. 6, B) no longer produce modifications in the activity of the respiratory centre.



A B

Fig. 6. Dog. 10·5 kg., chloralosane, depressor nerves cut. *R.*, pneumogram. *B.P.*, femoral arterial blood-pressure, mercury manometer. † *a*, common carotid arteries are clamped. Stimulation of the respiratory centre and increase of general blood-pressure with heart acceleration. † *b*, common carotid arteries are opened. Immediate inhibition of the respiratory activity and decrease of general blood-pressure with heart slowing. The two sinus caroticus nerves are then cut between A and B; this produces an increase of general blood-pressure (190 to 280 mm. Hg), heart acceleration and increase of rate and amplitude of respiration. † *c*, common carotid arteries are clamped. No changes in the activity of the respiratory centre, slight mechanical increase of general blood-pressure. † *d*, common carotid arteries are opened. No changes in the activity of the respiratory centre, slight mechanical drop of genera blood-pressure.

These experiments furnish evidence that the immediate respiratory responses to changes in cephalic blood-pressure and blood flow are due

only to reflexes from the sinus caroticus. The nerves of the sinus caroticus exert an inhibitory respiratory tonus which is maintained by the normal blood-pressure acting on the nerve endings of the sinus caroticus.

Adrenaline apnœa.

It is a well-known fact since the first experiments of Oliver and Schafer(22) that an intravenous injection of adrenaline produces a diminution in the rate and depth of respiration, sometimes a cessation of respiration, an adrenaline apnœa. In previous papers(1) we have shown that, in accordance with the conclusion of Voegtlin and Wiggers(23), the direct action of adrenaline on the respiratory centre is not to depress but to stimulate it. Evidence was also presented that adrenaline hypertension confined to the somatic circulation of a dog *B* produces a reflex (vagus-depressor) inhibition, an apnœa, of the respiratory centre of the isolated head of dog *B*, with its head perfused by means of a dog *A*. This reflex adrenaline apnœa is due to the increase of blood-pressure in the heart and aortic arch; if one prevents the rise of somatic blood-pressure no reflex apnœa of the isolated head occurs. We have concluded from these observations that the adrenaline apnœa is due essentially to a reflex (vagus-depressor) inhibition of the respiratory centre by the high blood-pressure. This conclusion was recently contradicted by C. F. Schmidt(24); for this worker "reflex inhibition appears to be a factor, but by far the most important cause is increase in blood supply of the centre." This conclusion is based upon the fact that "adrenaline is never made completely ineffective by vagotomy" and that "the brain of a cat was perfused *via* the vertebral arteries, addition of adrenaline to the perfusing blood caused marked rise in systemic pressure, but perfusion pressure was not raised and respiration was only very slightly depressed. However, when the carotids were opened, typical adrenaline apnœa occurred at once. The results demonstrate the dependence of adrenaline apnœa upon passive changes in cerebral blood flow and its independence of reflexes or of direct action upon the respiratory centre." But the experiments that we have described above indicate that the conclusions of Schmidt ought to be re-examined. In Schmidt's experiments, indeed, the adrenaline rise in blood-pressure does only produce a very slight respiratory depression (vagus depressor reflex) because the common carotid arteries were clamped, and this sets up a reflex (sinus caroticus) respiratory stimulation. However, when the carotids were opened, apnœa occurred at once, because the high systemic blood-pressure is acting on the nerve endings of the sinus caroticus. In

our experiments adrenaline hypertension, acting only on the two isolated sinus caroticus and with no increase but decrease in the cerebral blood flow, reproduces the same apnœa, which consequently is a reflex. This conclusion is also supported by the following experiments.

In dogs anæsthetized with urethane or chloralosane, the two depressor and sinus nerves are cut. Pneumogram (*R.*, Fig. 7) and femoral

Fig. 7. Dog. 11·0 kg., urethane, depressor and sinus caroticus nerves cut. *R.*, pneumogram. *B.P.*, arterial femoral blood-pressure, mercury manometer. †, intravenous injection of 0·3 mg. adrenaline. Hypertension but no changes in respiratory activity.

blood-pressure (*B.P.*, Fig. 7) are registered. The intravenous injection (†, Fig. 7) of 0·3 mg. adrenaline does not produce the slightest changes in respiratory rate or amplitude.

These experiments demonstrate that after section of the cardio-aortic nerves and sinus caroticus nerves, the adrenaline hypertension brings about neither inhibition nor cessation of respiratory activity, nor a slowing of the heart, although the cerebral blood flow and blood-pressure are increased.

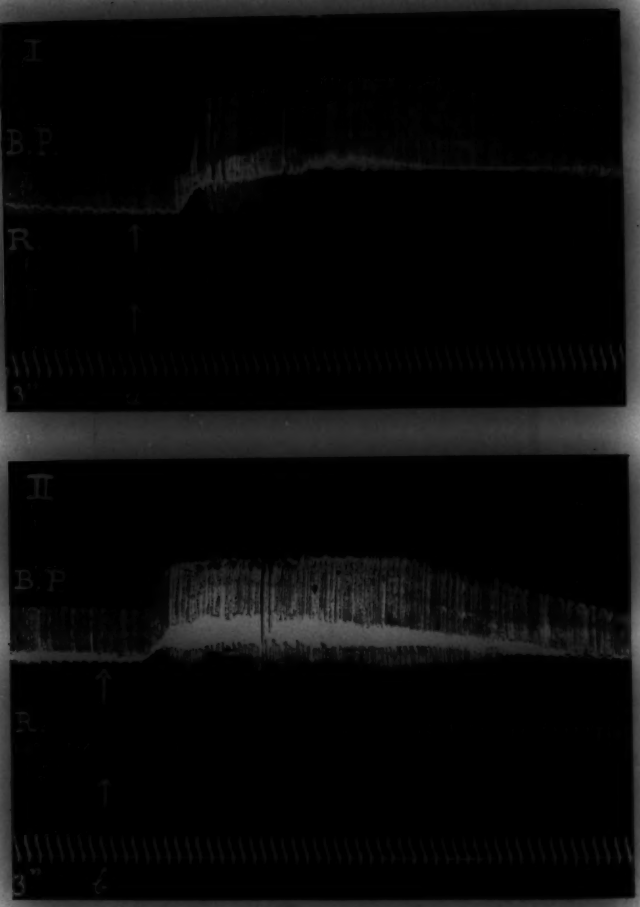


Fig. 8. Dog, 16 kg., chloralosane. Depressor nerves cut. *R.*, pneumogram. *B.P.*, femoral blood-pressure, tonometer. I, ↑*a*, intravenous injection of 0·2 mg. adrenaline. Hypertension; heart slowing and respiratory inhibition (apnœa). Between I and II the two sinus carotici are denervated. II, ↑*b*, intravenous injection of 0·2 mg. adrenaline. Hypertension; no heart slowing (vagi intact), slight inhibition of respiration followed by respiratory stimulation.

These facts should also be compared with the observations of McDowall(25) that "adrenaline apnœa is produced in a manner similar to and coincident with the vagal inhibition of the heart caused by adrenaline," since we know that the heart slowing and respiratory inhibition produced by an increase of blood-pressure are reflexes of cardio-aortic and sinus caroticus origin.

In some experiments (Fig. 8) one may observe that, in dogs with sinus and depressor nerves cut, the sudden increase of blood-pressure may produce a very slight depression of respiration; this inhibition of short duration is followed by increase of respiratory activity and also appears after adrenaline injection in dogs with carotid and vertebral arteries clamped and depressor and sinus nerves cut, therefore this slight inhibition must be a medullary afferent respiratory reflex.

Summary and conclusions.

1. A method of perfusion, with the Dale-Schuster pump, of the two isolated sinus caroticus, with nerve supply intact, is described.

2. The increase of blood-pressure in the isolated sinus caroticus circulation as well as in the cardio-aortic circulation produces a reflex inhibition of the respiration, a reflex apnœa.

3. A decrease of blood-pressure in the same vascular areas produces a reflex stimulation of respiratory activity, a reflex hyperpnœa.

4. Evidence is presented that the immediate changes in the activity of the respiratory centre, as well as the changes in heart rate and vasomotor tonus produced by modifications of blood-pressure and blood flow in the cephalic circulation, are due to reflexes arising in the sinus caroticus and not to modifications in the central blood supply. After denervation of the two sinus caroticus, occlusion of the carotids no longer produces respiratory reactions.

5. A humoral central apnœa or hyperpnœa may be transformed into a reflex hyperpnœa or into a reflex apnœa by lowering or increasing the blood-pressure only in the sinus caroticus or in the heart and aortic arch.

6. Adrenaline apnœa is due to a reflex inhibition of the respiratory centre produced by the increase of blood-pressure in the cardio-aortic and sinus caroticus circulatory areas and not to changes in the central blood supply.

7. The afferent nerves for these respiratory reflexes are the vagus-depressor nerves and the sinus caroticus nerves. In normal circulatory

conditions these nerves exert an inhibitory tonus on the activity of the respiratory centre.

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STUDIES IN MUSCULAR ACTIVITY.

VI. Response of several individuals to a fixed task.

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Introduction.

Experience teaches that individuals are not equal in physical ability. From prehistoric times contests in war and in sport have given convincing evidence of this fact. The question as to how one individual can surpass another in capacity and in skill is at least partially susceptible of analysis in the laboratory. This has been demonstrated by Hill and his associates (1), and by many others.

There remain, however, many unanswered questions raised by the rearguard runner. Does he fall behind because of inadequate physical endowment or because of insufficient training? His coach may ascribe his failure to his musculature, to his heart, to his "wind" or to his "guts." Since the empirical observations of the runner and his coach certainly have an objective foundation, it has been our aim to seek a basis for these ideas in physiological and physico-chemical facts.

Experimental.

Ten men, normal but not in active training, have performed the same task under controlled conditions. Each came to the laboratory without breakfast and rested for 30 minutes on a bed. With the subject in this state, respiratory and pulse rates were observed, expired air was collected in a 100 litre gasometer over a 10 minute period and then samples of alveolar air and "virtual venous" air were collected. Gas samples were analysed on the Haldane apparatus.

The method used for estimating blood flow is in basic principle that of Christiansen, Douglas and Haldane (2). Y. Henderson and Prince (3) modified it in important details and introduced the term "virtual venous" air. Field, Bock, Gildea and Lathrop (4) added further modifications. It is now necessary to modify it still further, for recent determinations (5) indicate that Haldane-Priestley samples collected in rest at the end of expiration have, without correction, the same pressure of carbonic acid as that in arterial

blood. Accordingly the carbonic acid pressures of alveolar air and of "virtual venous" air are applied directly to the carbonic acid dissociated curve of oxygenated whole blood. The difference between the indicated contents, diminished by 0·3 volume p.c. on the assumption that arterial blood is 95 p.c. saturated, represents carbon dioxide transport in volumes p.c. Rate of dioxide output divided by rate of transport gives rate of blood flow.

Of course this method for determining rate of blood flow, being indirect, is unsatisfactory. Yet many arguments of the kind commonly advanced for indirect methods may be presented in its defence. It yields results, particularly in exercise, of the same order of magnitude as those obtained by other methods; results in certain pathological conditions are qualitatively similar to blood velocity determinations[1]; repeated observations on a given individual in the same state give good checks; wholly improbable results are not obtained. So long as the evidence for the absolute accuracy of this and other indirect methods is of such a character, estimates of rate of blood flow must be accepted with reserve.

When all these observations were completed, about 65 c.c. of venous blood was drawn without stasis: 5 c.c. was set aside for serum calcium determination and the remainder was treated with heparin. Determinations made on blood will be described below.

Within an hour of the time resting blood was drawn, the subject, still fasting, began exercise. This consisted in running for 20 minutes on a treadmill, motor-driven in a horizontal plane at a rate of 9·3 km. per hour. Outside air was inspired through a large-size Henderson-Haggard valve (6) and alveolar air samples were collected automatically, since it is not practicable in exercise of this character to use the heavy system of valves suitable in rest or in exercise on the ergometer. The subject breathed continuously through the mouthpiece from the start until the 9th minute, and again from the 12th through the 17th minute. Other details, such as the method of collecting "virtual venous" air in exercise of this sort, are given by Hochrein, Talbott, Dill and Henderson (7).

Expired air was measured in a 600 litre gasometer, and six samples were taken by the mixing chamber method as described by Bock, Dill and Talbott (8). Pulse rate was recorded by a cardiotachometer which will be described by Paul S. Bauer, its designer, in detail at another time. It resembles in many particulars the instrument of Boas (9). The nature

[1] Reference may be made to the series of papers by H. L. Blumgart and Soma Weiss, *J. Clin. Inv.* 4. 15 (1927) *et seq.*, in which velocity was measured by injecting radioactive material and detecting its arrival at a distant point. The recent velocity measurements by Soma Weiss, G. P. Robb and H. L. Blumgart, *Amer. Heart J.* 4. 1 (1929), involved use of the effect of histamine on the minute vessels and gave similar results. W. O. Thompson, J. M. Alper and P. K. Thompson, *J. Clin. Inv.* 5. 605 (1928), have demonstrated posture effects on velocity from ankle to arm by the dye injection method; and A. V. Bock and associates, in an investigation in the course of publication, have obtained similar results with the histamine method. All the above experiments on blood velocity agree qualitatively with our determinations of rate of blood flow.

of other observations made during exercise will be disclosed in tables of data.

After running for 20 minutes the subject jumped on to the bed, and another 65 c.c. portion of venous blood was withdrawn. A sample of blood obtained within 1 minute of cessation of work of this intensity represents blood circulating during work in nearly every respect, except for different contents of oxygen and of carbonic acid. This is borne out by unpublished observations made in this laboratory on oxygen capacity, carbonic acid capacity and lactic acid content of blood taken during the course of, and within 1 minute of, cessation of exercise on the bicycle ergometer.

Each of the 60 c.c. portions of blood treated with heparin was kept in ice water until equilibration. Two 5 c.c. portions were removed for determination of lactic acid and oxygen combining capacity. The remainder was divided into a 45 c.c. and a 5 c.c. portion and equilibrated at 37·5° with sufficient oxygen for saturation at carbon dioxide pressures of about 40 and 80 mm. respectively. After 15 minutes' equilibration, blood was drawn into sampling tubes for carbon dioxide determination and about 40 c.c. of the larger portion, without exposure to air, were divided between two calibrated centrifuge tubes of 20 c.c. capacity. This technique and other determinations carried out on serum and cells have been described in detail by Dill, Talbott, Edwards and Oberg(10). The only new method used was Van Slyke's micro total nitrogen method(11). Single 10 c.c. portions of equilibrated serum and cells were dried to constant weight for total solid determination, and then ashed and used for sodium and potassium determination. Previous determinations of sodium and of potassium carried out in duplicate, using the same method(10), usually agreed within 1 mEq. per litre. In these experiments determinations were not carried out in duplicate, but rest and work serum and cells were analysed side by side, and it is likely that a difference between rest and work greater than 1 mEq. per litre is significant.

Table I records ventilation, pulse rate, respiratory rate, composition of expired air, metabolic rate (M.R.) and respiratory quotient (R.Q.). Composition of alveolar air and carbon dioxide pressure in gas samples equilibrated *in vivo* with oxygenated venous blood are shown in Table II. Four samples of each were taken in rest, and only the averages of these are shown. Alveolar samples were collected at about the 4th, 8th, 13th and 17th minutes in work, and each determination is given in order to show change of state if such occurred. Two "virtual venous" samples

TABLE I. Metabolic rate, respiration, and pulse.

Time (min.)	Remarks	Total ventilation (l. per min.)	Composition of expired air		Oxygen used (c.c. per min.)	Respiratory quotient	Pulse rate	Respiratory rate
			CO_2 (p.c.)	O_2 (p.c.)				
	D. B. D. Basal	5·38	3·77	16·53	246	0·82	62	10
0	Work begun							
4–5		41	4·43	15·95	2100	0·86	—	—
6–7		41	4·44	15·99	2080	0·87	138	21
12–13		48	4·17	16·46	2180	0·91	140	24
14–15		50	4·10	16·50	2260	0·90	135	22
16–17		45	4·02	16·53	2020	0·89	138	22
	P. F. P. Basal	7·90	2·65	17·58	277	0·75*	68	15
0	Work begun							
2–3		45	4·12	16·64	2180	0·95	—	37
4–5		52	4·09	16·89	2100	1·01	172	38
6–7		53	4·06	16·91	2130	1·00	164	44
14–15		67	3·81	17·10	2570	0·98	166	46
16–17		62	3·78	17·15	2350	0·99	164	45
17–18		62	3·72	17·01	2470	0·93	—	47
	A. A. McC. Basal	5·23	3·23	16·77	276	0·72*	50	15
0	Work begun							
3–4		65	4·23	16·55	2880	0·95	—	36
4–5		64	4·35	16·43	2900	0·95	—	—
6–7		59	4·29	16·22	2830	0·88	—	—
13–14		59	4·27	16·09	2950	0·85	—	34
15–16		59	4·45	15·88	3080	0·85	132	—
17–18		58	4·42	16·05	2900	0·88	132	36
	J. H. T. Basal	5·89	3·33	16·94	242	0·80	64	9
0	Work begun							
3–4		50	3·84	16·60	2220	0·85	153	—
5–6		52	3·75	16·90	2130	0·90	153	25
7–8		53	3·72	17·03	2100	0·93	153	—
11–12		54	3·70	17·04	2150	0·93	153	—
13–14		52	3·76	16·91	2120	0·91	153	37
16–17		53	3·57	17·15	2020	0·92	153	36
	W. C. Basal	7·23	3·35	16·90	305	0·79	69	15
0	Work begun							
3–4		63	4·68	16·45	2790	1·04	174	41
5–6		71	4·06	17·06	2720	1·05	168	—
7–8		70	3·91	16·90	2850	0·95	174	—
13–14		74	3·93	17·06	2850	1·01	174	42
15–16		80	3·63	17·38	2830	1·02	180	—
16–17		82	3·45	17·60	2710	1·03	176	49
	O. S. L. Basal	5·94	3·39	16·96	246	0·81	71	12
0	Work begun							
2–3		44	4·93	15·63	2380	0·91	144	14
4–5		45	5·19	15·28	2600	0·90	146	—
7–8		46	5·13	15·48	2560	0·92	160	12
14–15		49	4·86	15·71	2610	0·91	163	15
15–16		50	4·74	15·95	2530	0·93	156	18
16–17		51	4·69	16·04	2520	0·94	159	19

* Probably a fallacious value due to abnormal breathing.

TABLE I. Metabolic rate, respiration, and pulse (*continued*).

Time (min.)	Remarks	Total ventilation (l. per min.)	Composition of expired air: CO_2 (p.c.)	Composition of expired air: O_2 (p.c.)	Oxygen used (c.c. per min.)	Respiratory quotient	Pulse rate	Respiratory rate
	J. L. S. Basal	6·12	3·22	17·40	222	0·88*	69	14
0	Work begun							
3–4		53	4·59	16·22	2520	0·96	—	25
5–6		55	4·30	16·51	2450	0·95	—	—
7–8		53	4·36	16·39	2460	0·94	—	29
11–12		54	4·34	16·40	2470	0·94	—	30
14–15		53	4·25	16·41	2420	0·92	—	—
17–18		53	4·12	16·47	2390	0·90	—	30
	H. T. E. Basal	4·15	4·32	15·66	232	0·78	66	10
0	Work begun							
3–4		35	5·24	14·91	2180	0·84	153	15
5–6		39	4·80	16·95	1950	0·95	154	—
7–8		42	4·77	15·75	2220	0·90	156	19
12–13		46	4·50	16·15	2210	0·92	154	21
14–15		44	4·62	15·90	2260	0·90	156	—
16–17		43	4·69	15·58	2380	0·84	—	20
	A. V. B. Basal	4·90	4·02	16·26	237	0·82	61	5
0	Work begun							
2–3		47	4·03	16·41	2190	0·86	—	21
5–6		52	4·02	16·80	2170	0·96	142	—
7–8		55	3·96	16·60	2440	0·88	144	32
11–12		60	3·87	16·92	2430	0·95	144	33
14–15		57	3·72	16·98	2290	0·92	—	—
17–18		56	3·75	16·78	2380	0·87	142	33
	W. J. G. Basal	5·02	3·60	16·83	212	0·83	62	14
0	Work begun							
2–3		55	4·16	16·78	2280	1·00	156	27
3–4		55	4·12	16·75	2290	0·97	170	—
7–8		55	3·92	16·90	2230	0·96	168	31
12–13		55	3·82	16·82	2300	0·91	168	30
16–17		56	3·99	16·80	2340	0·95	169	31

* Probably a fallacious value due to abnormal breathing.

TABLE II. Determination of blood flow.

Remarks	Alveolar air: Respiratory quotient	Alveolar air: (pCO_2 mm. Hg)	Alveolar air: (pO_2 mm. Hg)	Virtual venous air (pCO_2 mm. Hg)	CO_2 transport (mEq. per l. blood)	O_2 transport (mEq. per l. blood)	O_2 saturation of venous blood (p.c.)	Blood flow (l. per min.)
D. B. D. Basal	0·82	40·2	105	47·6	1·35	1·65	75	6·7
Work	0·89	42·0	106	—	—	—	—	—
	0·94	40·2	110	65·4	4·44	4·99	38	18·9
	0·92	39·4	110	—	—	—	—	—
	0·92	40·5	109	62·7	3·95	4·44	44	21·3
Average Δ	+0·10	+ 0·3	+ 4	+16·5	+2·85	+3·07	−34	+13·4
P. F. P. Basal	0·82	36·3	106	43·3	1·30	1·73	75	7·1
Work	0·96	39·8	108	71·5	4·72	4·82	41	21·1
	0·97	37·4	111	—	—	—	—	—
	0·88	36·8	109	66·8	5·12	5·22	37	19·4
Average Δ	+0·12	+ 1·7	+ 3	+25·8	+3·62	+3·29	−36	+13·1

TABLE II. Determination of blood flow (*continued*).

Remarks		Alveolar air: Respiratory quotient	Alveolar air: (pCO_2 mm. Hg)	Alveolar air: (pO_2 mm. Hg)	Virtual venous air (pCO_2 mm. Hg)	CO_2 transport (mEq. per l. blood)	O_2 transport (mEq. per l. blood)	O_2 saturation of venous blood (p.c.)	Blood flow (l. per min.)
A. A. McC.	Basal	0·81	38·0	103	46·1	1·53	2·12	69	5·9
	Work	0·88	42·3	102	—	—	—	—	—
		0·81	42·1	99	67·0	4·23	4·75	43	27·3
		0·83	42·0	100	—	—	—	—	—
		0·87	40·3	103	69·7	5·00	5·61	34	23·1
Average Δ		+0·04	+ 3·7	− 2	+22·3	+3·09	+3·06	−31	+19·3
J. H. T.	Basal	0·80	39·5	103	45·8	1·12	1·40	80	8·8
	Work	0·86	37·2	112	—	—	—	—	—
		0·95	35·5	112	62·6	4·81	5·28	46	17·9
		0·92	34·3	112	—	—	—	—	—
		0·85	34·3	110	60·1	4·63	5·09	48	18·7
Average Δ		+0·09	− 4·2	+ 8	+15·6	+3·60	+3·79	−33	+ 9·5
W. C.	Basal	1·02	35·6	113	45·0	1·65	2·09	72	6·7
	Work	1·05	45·2	105	—	—	—	—	—
		0·99	37·3	110	75·1	5·48	5·32	35	24·4
		1·00	32·7	116	72·9	—	—	—	—
		1·01	33·5	115	—	5·66	5·50	33	21·8
Average Δ		−0·01	+ 1·6	− 2	+29·0	+3·92	+3·32	−38	+16·4
O. S. L.	Basal	1·11	38·2	114	45·0	1·12	1·38	79	8·0
	Work	0·84	42·9	101	—	—	—	—	—
		0·87	45·4	99	67·8	3·64	3·96	52	28·8
		0·86	43·4	102	—	—	—	—	—
		0·87	42·1	103	66·3	4·00	4·35	48	26·3
Average Δ		−0·25	+ 5·2	− 13	+22·1	+2·70	+2·78	−29	+19·6
J. L. S.	Basal	1·06	39·3	111	46·6	1·44	1·63	78	6·0
	Work	0·94	42·0	106	—	—	—	—	—
		1·07	40·6	104	70·9	5·00	5·38	41	20·7
		0·92	39·7	107	—	—	—	—	—
		0·92	39·7	107	67·7	4·76	5·12	44	20·8
Average Δ		−0·11	+ 1·2	− 5	+22·7	+3·44	+3·62	−36	+14·8
H. T. E.	Basal	0·72	40·3	98	46·8	1·15	1·47	78	7·4
	Work	0·78	47·0	94	—	—	—	—	—
		0·61	43·2	87	69·7	4·22	4·74	44	21·2
		0·85	41·0	103	—	—	—	—	—
		0·81	42·1	100	69·3	4·42	4·96	42	20·4
Average Δ		+0·04	+ 3·0	− 2	+22·7	+3·17	+3·38	−35	+13·4
A. V. B.	Basal	0·87	38·1	100	45·9	1·39	1·70	76	6·3
	Work	0·88	38·4	102	—	—	—	—	—
		0·87	38·0	102	63·0	4·59	4·99	45	20·7
		0·88	34·7	106	—	—	—	—	—
		0·86	34·7	105	60·3	4·86	5·28	42	19·5
Average Δ		0·00	− 1·7	+ 4	+15·8	+3·34	+3·44	−33	+13·8
W. J. G.	Basal	1·04	43·7	108	49·5	1·04	1·24	82	7·8
	Work	0·90	38·6	109	—	—	—	—	—
		0·94	37·8	111	66·2	5·16	5·43	43	19·1
		0·92	35·7	112	—	—	—	—	—
		0·88	36·9	110	59·7	4·55	4·79	49	21·6
Average Δ		−0·13	− 6·5	+ 1	+13·6	+3·82	+3·87	−36	+12·5

TABLE III. Experimental observations on oxygenated blood.

Blood equilibrated with carbonic acid

Serum calcium	(Total CO_2	(CO_2	(H_2O	(H_2O	(Na	(Na	(E	$\frac{(HPO_4)^{--}}{(H_2PO_4)^-}$

TABLE III. Experimental observations on oxygenated blood.

			Blood equilibrated with carbonic acid												
Subject		Serum calcium (mEq. per l. serum)	pCO_2 (mm. Hg)	(Total CO_2)$_b$ (mEq. per l. blood)	(Total CO_2)$_s$ (mEq. per l. serum)	Cell vol. (p.c.)	(Cl)$_s$ (mEq. per l. serum)	(Cl)$_c$ (mEq. per l. cells)	(H_2O)$_s$ (c.c. per l. serum)	(H_2O)$_c$ (c.c. per l. cells)	(Na)$_s$ (mEq. per l. serum)	(Na)$_c$ (mEq. per l. cells)	(K)$_s$ (mEq. per l. serum)	(K)$_c$ (mEq. per l. cells)	$(HPO_4)_s^{--+}$ $(H_2PO_4)_s^{-}$ (mEq. per l. serum)	(Protein)$_s$ (g. per l. serum)
D. B. D.	Rest	4·81	58·6	25·91	30·86	42·0	103·3	56·1	940	734	137·5	16·7	3·2	93·5	2·29	70·8
			82·0	29·50												
	Work	5·05	46·7	22·28	26·98	44·1	103·7	55·7	934	728	138·0	15·8	3·7	96·4	2·57	79·9
			90·4	29·20												
P. F. P.	Rest	—	39·1	21·73	26·40	45·7	104·4	54·7	940	730	137·0	14·5	3·6	95·7	2·18	70·0
			68·4	27·00												
	Work	—	30·5	15·34	18·56	47·6	106·2	56·2	933	726	141·6	13·2	3·0	96·3	2·57	77·1
A. A. McC.	Rest	4·58	42·4	21·84	26·67	44·5	107·0	54·0	939	727	—	—	—	—	2·48	—
			75·2	27·70												
	Work	4·82	39·0	19·46	23·98	47·9	107·6	54·7	931	725	—	—	—	—	2·92	—
			81·1	26·60												
J. H. T.	Rest	4·69	42·4	21·42	26·11	46·2	105·7	52·5	940	725	139·0	15·0	2·9	93·6	1·79	68·6
			58·6	24·38												
	Work	4·93	42·7	21·01	25·95	47·8	106·0	52·9	939	724	140·7	14·4	2·7	94·3	2·07	71·6
			99·9	30·05												
W. C.	Rest	4·75	44·3	22·22	26·70	44·4	105·2	53·3	937	724	134·5	15·4	2·9	88·7	2·46	72·0
			60·9	24·93												
	Work	5·05	42·7	15·93	18·63	45·8	106·1	58·0	933	725	140·5	16·0	2·8	90·4	3·30	77·7
			60·9	18·77												
O. S. L.	Rest	4·55	44·8	22·40	26·81	41·8	108·1	53·7	939	722	139·8	17·2	3·2	90·6	2·20	71·4
			56·7	25·49												
	Work	4·70	43·4	18·97	22·68	44·9	107·8	56·1	932	722	142·6	17·4	2·8	90·2	2·72	78·0
			68·3	23·31												
J. L. S.	Rest	4·70	43·1	20·82	26·05	48·3	106·3	52·6	938	726	141·5	13·1	3·6	96·8	2·14	69·4
			88·2	28·30												
	Work	4·70	37·5	18·94	23·38	49·4	107·5	53·6	935	724	138·5	13·3	3·7	96·8	2·80	73·9
			84·0	26·57												
H. T. E.	Rest	4·65	42·6	21·67	26·29	44·1	106·4	51·9	940	729	140·3	13·5	2·9	99·1	2·34	69·4
			67·8	26·00												
	Work	4·85	38·8	17·43	21·04	46·7	107·7	57·9	934	727	143·5	13·5	3·3	98·2	2·87	77·0
			69·2	22·55												
A. V. B.	Rest	4·45	33·8	19·57	23·79	43·9	107·0	52·2	939	726	138·5	14·7	4·3	92·6	2·30	65·0
			93·7	29·22												
	Work	4·75	40·3	18·85	23·39	48·8	104·8	53·8	929	718	139·0	12·5	4·2	92·6	2·57	78·8
			68·6	24·02												
W. J. G.	Rest	—	45·1	21·92	26·42	44·3	107·1	51·1	945	718	140·2	14·1	3·1	92·7	1·88	62·5
			68·8	26·09												
	Work	4·65	39·9	18·66	23·13	47·4	108·0	51·9	939	715	143·3	13·3	2·9	93·4	2·55	71·2
			69·6	24·14												

were collected during the 9th to 12th minutes, and two more during the 18th to 20th. The average of each pair is given. Values for carbon dioxide transport and rate of blood flow, calculated from the data of Tables I and II, are also given in Table II. Experimental observations on blood equilibrated with oxygen and carbon dioxide are given in Table III.

Under the conditions of our experiments it was not practicable to secure arterial blood nor to equilibrate venous blood at precisely the carbonic acid pressure of arterial blood. From our knowledge of shift of anions and water between cells and plasma with change in acidity and from the present experimental observations, it is possible to recalculate the data to a basis more convenient for comparison.

Thus it is possible to recalculate to the basis of (*a*) a given pH_s value; (*b*) a given carbonic acid pressure, say 40 mm. Hg; (*c*) the estimated carbonic pressure of arterial blood; or (*d*) the estimated carbonic acid pressure of mixed venous blood.

Consideration of these procedures brings to mind the discussion by L. J. Henderson (12) of corresponding states. Any attempt to bring two or more individuals, or even specimens of their blood, to the same state in all probability will fail because of the interdependence of the variables involved. When a given variable has been brought to a common value others will be disturbed. This is well illustrated in the present case. Thus we may bring all specimens of blood to the same pH_s value for rest and work. Calculated values for pH of arterial serum in rest and work are shown in Table IV. These calculations involve the assumptions that

TABLE IV. pH of arterial serum in rest and at the end of work.

Subject	pH_s Rest	pH_s Work	ΔpH_s	Ventilation per kg. body weight (litres)
D. B. D.	7·42	7·39	−0·03	0·58
P. F. P.	7·44	7·32	−0·12	0·80
A. A. McC.	7·42	7·37	−0·05	0·72
J. H. T.	7·40	7·44	+0·04	0·67
W. C.	7·44	7·29	−0·15	1·19
O. S. L.	7·41	7·31	−0·10	0·67
J. L. S.	7·39	7·37	−0·02	0·72
H. T. E.	7·39	7·30	−0·09	0·59
A. V. B.	7·40	7·40	0·0	0·70
W. J. G.	7·36	7·38	+0·02	0·81
Average...	7·41	7·36	−0·05	0·75

arterial blood is 95 p.c. saturated in work of this character, and that alveolar carbon dioxide pressure measures accurately arterial carbon

dioxide pressure. Arterial punctures in exercise reported by Dill, Laurence, Hurxthal and Bock(13) fully justify these assumptions when one is considering the average of a series of observations on the ergometer but such general agreement does not ensure that every individual observation is correct. The values in Table IV show clearly that, on the average, there is a decrease in pH of arterial serum of about 0·05. However, it is equally clear that some individuals show slight change and others large change in $p\mathrm{H}_s$. This variation as well as related physiological changes would be masked by comparing properties of blood in rest and work at a common $p\mathrm{H}_s$ value.

TABLE V. Carbon dioxide pressure in alveolar air in rest and work.

Subject	Rest (mm. Hg)	Work		Δ $p\mathrm{CO}_2$ from rest to end of work (mm. Hg)
		Average (mm. Hg)	End of work (mm. Hg)	
D. B. D.	40·2	40·5	40·5	+0·3
P. F. P.	36·3	38·0	36·8	+0·5
A. A. McC.	38·0	41·7	40·3	+2·3
J. H. T.	39·2	37·4	37·1	−2·1
W. C.	36·4	37·3	33·5	−2·9
O. S. L.	38·2	43·4	42·1	+3·9
J. L. S.	39·3	40·5	39·7	+0·4
H. T. E.	40·3	43·4	42·1	+1·8
A. V. B.	38·1	36·4	34·7	−3·4
W. J. G.	43·7	37·2	36·9	−6·8
Average...	39·0	39·6	38·4	−0·6
Median ...	38·7	39·7	38·2	+0·3

Table V has been compiled in order to test the second method of recalculation suggested above. Two columns are given for work. One contains an average of all four samples taken during work and the other gives values for the last work sample only. It appears that in exercise of this character (*a*) there is not usually much change in alveolar $p\mathrm{CO}_2$ from rest to work nor during progress of work, and (*b*) the changes which do occur have no distinct relation to degree of lactic acid accumulation. The evidence for the latter statement will be given later.

Accordingly this method of recalculation was finally decided upon, viz. on the basis of a carbonic acid pressure of 40 mm. Hg. While open to some objection, it involves no doubtful assumptions, it is simple and it gives values at least approximately the same as if arterial blood had been used; in short, it gives values approximately of the order of corresponding states. The recalculations for serum are given in Table VI and for whole blood in Table VII.

TABLE VI. Electrolytes of true plasma at a carbonic acid pressure of 40 mm. Hg.
Concentrations are expressed in mEq. per litre of serum, excepting hydrogen ion.

Subject		(Total CO_2)	*p*H	Serum vol. (p.c.)	$(HCO_3)^-$	$(Lactate)^-$	$(Cl)^-$	$(HPO_4)^{--}$ + $(H_2PO_4)^-$	$(Protein-ate)^-$	Σ anions	Σ cations*
D. B. D.	Rest	27·20	7·42	58·5	25·96	1·0	104·0	2·3	17·4	150·7	147·5
	Work	25·62	7·40	56·1	24·38	1·8	104·4	2·6	19·5	152·7	148·8
	Δ	−1·58	−0·02	−2·4	−1·58	+0·8	+0·4	+0·3	+2·1	+2·0	+1·3
P. F. P.	Rest	26·57	7·41	54·3	25·33	1·1	104·4	2·2	17·1	150·1	147·3
	Work	20·68	7·30	52·2	19·44	5·8	105·2	2·6	18·0	151·0	151·6
	Δ	−5·89	−0·11	−2·1	−5·89	+4·7	+0·8	+0·4	+0·9	+0·9	+4·3
J. H. T.	Rest	25·58	7·40	53·9	24·34	1·6	107·2	1·8	16·7	151·6	148·6
	Work	25·24	7·39	52·3	24·00	2·1	107·6	2·1	17·4	153·2	150·3
	Δ	−0·34	−0·01	−1·6	−0·34	+0·5	+0·4	+0·3	+0·7	+1·6	+1·7
W. C.	Rest	26·08	7·40	55·7	24·84	1·3	105·7	2·5	17·5	151·9	144·2†
	Work	18·12	7·24	54·3	16·88	7·2	106·2	3·3	17·5	151·1	150·4
	Δ	−7·96	−0·16	−1·4	−7·96	+5·9	+0·5	+0·8	0·0	−0·8	
O. S. L.	Rest	26·08	7·40	58·2	24·84	1·3	108·7	2·2	17·4	154·4	149·6
	Work	22·59	7·34	55·2	21·35	3·7	108·2	2·7	18·5	154·4	152·1
	Δ	−3·49	−0·06	−3·0	−3·49	+2·4	−0·5	+0·5	+1·1	−0·0	+2·5
J. L. S.	Rest	25·13	7·39	51·8	23·89	1·3	106·6	2·1	16·8	150·7	151·8
	Work	23·96	7·37	50·5	22·72	2·1	107·3	2·8	17·7	152·6	146·9†
	Δ	−1·17	−0·02	−1·3	−1·17	+0·8	+0·7	+0·7	+0·9	+1·9	
H. T. E.	Rest	25·67	7·40	56·0	24·43	1·2	106·6	2·4	16·9	151·5	149·9
	Work	21·15	7·31	53·3	19·91	3·8	107·6	2·8	18·0	152·1	153·7
	Δ	−4·52	−0·09	−2·7	−4·52	+2·6	+1·0	+0·4	+1·1	+0·6	+3·8
A. V. B.	Rest	25·19	7·39	56·3	23·95	1·1	106·4	2·3	15·8	149·6	149·3
	Work	23·81	7·36	51·2	22·57	3·4	104·8	2·6	18·8	152·2	150·0
	Δ	−1·38	−0·03	−5·1	−1·38	+2·3	−1·6	+0·3	+3·0	+2·6	+0·7
W. J. G.	Rest	25·31	7·39	55·9	24·07	1·1	107·6	1·9	15·2	149·9	150·3
	Work	23·11	7·35	52·6	21·87	3·4	108·0	2·6	16·9	152·8	153·5
	Δ	−2·20	−0·04	−3·3	−2·20	+2·3	+0·4	+0·7	+1·7	+2·9	+3·2
Average,	Rest	25·87	7·40	55·6	24·63	1·2	106·1	2·2	16·7	151·2	149·3
	Work	22·70	7·34	53·1	21·46	3·7	106·3	2·7	18·0	152·5	151·3
	Δ	−3·17	−0·06	−2·5	−3·17	+2·5	+0·2	+0·5	+1·3	+1·3	+2·0

* Concentration of magnesium is assumed to be 2 mEq. per litre of serum. † Doubtful result. Not included in the average.

TABLE VII. Electrolytes of oxygenated blood at a carbonic acid pressure of 40 mm. Hg.
Concentrations are expressed in mEq. per litre of blood.

TABLE VII. Electrolytes of oxygenated blood at a carbonic acid pressure of 40 mm. Hg. Concentrations are expressed in mEq. per litre of blood.

Subject		Total Hb	Total CO_2	$(HCO_3)^-$	(Lactate)$^-$	$(Cl)^-$	$(HPO_4)^{--}$ + $(H_2PO_4)^-$	Protein-ate)$^-$	$(Na)^+$	$(K)^+$	Σ anions	Σ cations*
D. B. D.	Rest	8·27	22·44	21·30	0·80	83·5	1·7	30·1	86·8	41·1	137·4	132·9
	Work	8·76	21·00	19·86	1·47	82·5	1·9	31·6	84·1	44·6	137·3	133·7
	Δ	+0·49	−1·44	−1·44	+0·67	−1·0	+0·2	+1·5	−2·7	+3·5	−0·1	+0·8
P. F. P.	Rest	8·92	21·90	20·76	0·90	81·7	1·6	31·4	81·0	45·7	136·4	131·7
	Work	9·56	17·36	16·22	4·85	82·4	1·9	30·4	80·4	47·4	135·8	132·8
	Δ	+0·64	−4·54	−4·54	+3·95	+0·7	+0·3	−1·0	−0·6	+1·7	−0·6	+1·1
J. H. T.	Rest	9·34	20·82	19·68	1·30	81·1	1·4	31·2	81·7	45·8	134·7	132·5
	Work	9·86	20·33	19·19	1·74	80·6	1·6	32·2	80·3	46·5	135·3	131·8
	Δ	+0·52	−0·49	−0·49	+0·44	−0·5	+0·2	+1·0	−1·4	+0·7	+0·7	−0·7
W. C.	Rest	9·13	21·54	20·40	1·08	82·1	1·9	32·1	81·6†	41·0	137·6	127·6†
	Work	9·29	15·56	14·42	6·04	84·1	2·5	28·2	83·5	42·9	135·3	131·4
	Δ	+0·16	−5·98	−5·98	+4·96	+2·0	+0·6	−3·9	+1·9†	+1·9	−2·3	
O. S. L.	Rest	8·63	21·59	20·45	1·12	85·4	1·6	30·6	88·6	39·7	139·2	133·3
	Work	9·29	18·36	17·22	3·18	84·6	2·0	29·9	86·3	42·0	136·9	133·3
	Δ	+0·66	−3·23	−3·23	+2·06	−0·8	+0·4	−0·7	−2·3	+2·3	−2·3	0·0
J. L. S.	Rest	9·79	20·23	19·09	1·08	80·4	1·6	31·6	79·5	48·6	133·8	133·1
	Work	10·09	19·56	18·42	1·74	81·9	2·1	32·6	76·6†	49·7	136·8	131·3†
	Δ	+0·30	−0·67	−0·67	+0·66	+1·5	+0·5	+1·0	−2·9†	+1·1	+3·0	
H. T. E.	Rest	8·92	21·15	20·01	0·99	82·3	1·7	30·7	84·4	44·3	135·7	133·7
	Work	9·41	17·60	16·46	3·13	84·4	2·1	30·6	82·7	47·6	136·7	135·3
	Δ	+0·49	−3·55	−3·55	+2·14	+2·1	+0·4	−0·1	−1·7	+3·3	+1·0	+1·6
A. V. B.	Rest	8·91	20·94	19·77	0·90	82·9	1·7	30·0	84·1	43·1	135·3	132·2
	Work	9·92	18·80	17·66	2·87	79·9	1·9	31·1	77·3	47·3	133·4	129·6
	Δ	+1·01	−2·11	−2·11	+1·97	−3·0	+0·2	+1·1	−6·8	+4·2	−1·9	−2·6
W. J. G.	Rest	9·47	20·81	19·67	0·95	82·3	1·4	30·6	84·3	42·8	134·9	132·1
	Work	10·50	18·70	17·56	2·87	81·4	1·9	31·9	81·7	45·8	135·6	132·5
	Δ	+1·03	−2·11	−2·11	+1·92	−0·9	+0·5	+1·3	−2·6	+3·0	+0·7	+0·4
Average,	Rest	9·04	21·27	20·13	1·01	82·3	1·6	30·9	84·4	43·6	136·0	132·7
	Work	9·63	18·61	17·47	3·10	82·3	2·0	30·9	81·8	46·0	135·8	132·6
	Δ	+0·59	−2·66	−2·66	+2·09	0·0	+0·4	0·0	−2·6	+2·4	−0·2	−0·1

* Concentration of calcium plus magnesium in whole blood is assumed to be 5 mEq. per litre in rest and in work.
† Doubtful result. This value is not used in calculating the average.

Interpretation of results.

Concentration changes in protein of serum and hæmoglobin of blood. Zuntz with his associates(14) was perhaps the first to demonstrate clearly that increase in sp. gr. and cell count of blood takes place in exercise. In 28 experiments, marching with a pack of 22–31 kg. over a course of 18–24½ km. caused an increase in sp. gr. of 2 to 4 thousandths and in cell count of about 9 p.c. Later it was shown by Ferrari(15) that the average red cell count of students may increase 10 p.c. during the course of an examination. Since then much more evidence has accumulated that change in degree of physical activity or in emotional state is reflected by hæmoglobin concentration change in the blood.

In the cat suddenly confronted by the barking dog, there is an increase of 20 to 30 p.c. (or, rarely, 40 to 50 p.c.) in red cell count as shown by Lamson(16) and Izquierdo and Cannon(17). The latter authors, having at hand knowledge of the spleen as a reservoir of red cells recently acquired by Barcroft and his associates(18) and many others, demonstrated that when only the liver of the upper abdominal viscera is left innervated, there is no emotional polycythemia. Furthermore:

"After section of the nerves to the spleen, excitement for one minute does not produce polycythemia.... Inactivation of the adrenal medulla has no marked influence on emotional polycythemia."

Aside from contributions of polycythemic blood by the spleen, there may be a change due to salt solution transfer from blood to tissues or interstitial spaces. Imbibition by frog's muscle in strychnine tetanus and simultaneous loss of fluid by the blood was reported by Ranke(19) in 1865. Water content of blood of resting frogs was 88·3 p.c. and of tetanized frogs 87·0 p.c. Corresponding values for muscle were 80·3 and 82·1 respectively. More recently Back, Cogan and Towers(20), also working on frog's muscle, found that faradic stimulation caused increases in water content ranging from 0·7 to 7·6 p.c., averaging about 4 p.c.

Increase in hæmoglobin concentration in exercise varies with the species and with the conditions of exercise. For man Bock, van Caulært, Dill, Fölling and Hurxthal(21) found increases of 5 to 10 p.c. with some difference in individuals, the maximum increase of about 10 p.c. occurring in A. V. B. at a metabolic rate of about 2 litres of oxygen per minute. Increases found by Harrison, Robinson and Syllaba(22) and by Himwich and Loebel(23) were of the same order of magnitude. In contrast with these moderate changes in man are results on the horse reported by Scheunert and Krzywanek(24). During 5 minutes' trot,

six experiments showed increase in cell volume (expressed in p.c. of blood volume) from 30 to 40, 29 to 36, 28·7 to 38, 24 to 35·5, 25 to 37 and 29 to 39·5 p.c. respectively. The average increase was from 27·6 p.c. to 37·6 p.c. or about one-third.

In Scheunert and Krzywanek's experiments increase in plasma protein concentration was determined and associated decrease in plasma volume was estimated. Increase in p.c. cell volume by this water transfer represented but a small part of the total observed increase and it was concluded that the spleen must make a large contribution of cells to the active circulation.

In exercise experiments on man which caused sweating and lasted for 15 to 30 minutes, Cohn(25) found a decrease in hæmoglobin concentration. At the same time increase in plasma protein concentration took place. Cohnheim, Kreglinger and Kreglinger(26) as well as Gross and Kestner(27) found a decrease in red count and p.c. hæmoglobin after prolonged and strenuous mountain climbing. These latter observations we have confirmed in recent experiments but Cohn's result remains unexplained.

It was found by Broun(28) that after 10 or 15 minutes' exercise, dogs had an average increase of 3·6 p.c. in total plasma volume and 12·3 p.c. in total cell volume, the range of the latter increment being from 1·5 to 24 p.c. There was thus a distinct increase in circulating blood volume. After several hours' exercise both total cell volume and hæmoglobin content were less than after 10 minutes' exercise, a difference that was much greater in animals which had been confined for several months. The conclusion was reached that blood destruction is a significant phenomenon during prolonged exercise in untrained dogs. Results of a similar character have been obtained by Hastings(29).

Barcroft and Florey(30) have succeeded in resecting a portion of colon of the dog and sewing it, with blood supply still maintained, into the belly wall. Exercise and emotional disturbance result in a simultaneous decrease in size of the spleen and in the pink colour of the colon. A shutting down of vessels in the splanchnic area is clearly proved by these experiments.

Chiatellino and Margaria(31) have studied concentration changes in the blood of men during a march of 2 to 6 hours with no water intake. Loss of body weight ranged from 1·6 to 3·5 p.c. Increase was slight for osmotically active substances and marked for serum proteins and red cells. An actual diminution in blood volume was observed.

Finally we come to our own results. We have in Table VIII values

TABLE VIII. Effect on cell volume of concentration changes in serum and cells from rest to work.

A. Normal subjects.

Subject	Serum solids		Serum protein		Cell solids		Cell volume		
	Rest (g. per l.)	Work (g. per l.)	Rest (g. per l.)	Work (g. per l.)	Rest (g. per l.)	Work (g. per l.)	Rest (p.c.)	Work (observed p.c.)	Work (calculated p.c.)
D. B. D.	86·5	93·6	70·8	79·9	366	372	42·0	44·1	44·6
P. F. P.	86·9	94·2	—	—	370	374	45·7	47·6	47·4
A. A. McC.	86·4	97·1	—	—	375	378	44·5	47·9	47·2
J. H. T.	84·4	87·0	68·6	71·6	377	380	46·2	47·8	47·1
W. C.	88·4	94·2	72·0	77·7	380	377	44·4	45·8	46·5
O. S. L.	86·2	95·8	71·4	78·0	387	386	41·8	44·9	44·0
J. L. S.	88·1	92·4	69·4	73·9	376	378	48·3	49·4	49·7
H. T. E.	85·2	93·3	69·4	77·0	373	376	44·1	46·7	46·5
A. V. B.	90·8	99·8	65·0	78·8	377	389	43·9	48·8	47·9
W. J. G.	77·8	86·3	62·5	71·2	387	390	44·3	47·4	47·3
Average...	86·1	93·4	68·6	76·0	377	380	44·5	47·0	46·8
B. Subjects after splenectomy.									
W. K.	84·1	90·4	—	—	389	387	48·0	49·4	49·9
"	78·5	89·0	—	—	389	389	43·6	47·0	46·7
"	91·8	91·8	—	—	386	389	48·7	48·3	48·5
E. H.	91·0	100·5	—	—	372	373	48·2	51·4	50·7
Average...	86·4	92·9	—	—	384	385	47·1	49·0	49·0

for serum solids, serum protein, cell solids and p.c. cell volume in rest and work. In addition to the ten subjects already referred to, we were fortunate in securing observations on two young men without spleens. Splenectomy had been performed about two years before our observations on E. H. and five years before in the case of W. K. Recovery was complete in each case.

The last column in this table shows values for p.c. cell volume calculated as follows:

$$V_c' = 100 \left(\frac{V_c P_s}{P_s'}\right) \div \left[\left(\frac{V_c P_c}{P_c'}\right) + \left(\frac{[100 - V_c] P_s}{P_s'}\right)\right],$$

where V_c' = p.c. cell volume in work;
V_c = p.c. cell volume in rest;
P_c = g. of solids per litre of cells in rest;
P_c' = g. of solids per litre of cells in work;
P_s = g. of protein per litre of serum in rest;
P_s' = g. of protein per litre of serum in work.

This formula is a mathematical expression of the hypothesis that all the changes are due simply to loss of water from the blood, during work. The loss of water per unit weight of serum and of cells was determined by analysis but we have no information regarding total blood volume changes.

In most cases determinations of serum solids and of serum protein were made. The values for protein furnish the more suitable basis for recalculation and these values were used when available. Otherwise values for serum solids were employed. The result is essentially the same; the average calculated p.c. cell volume for normal men comes out 46·8 on the basis of serum protein concentration change, and 46·6 on the basis of serum solids change. This fact is expressed in another form in Fig. 1

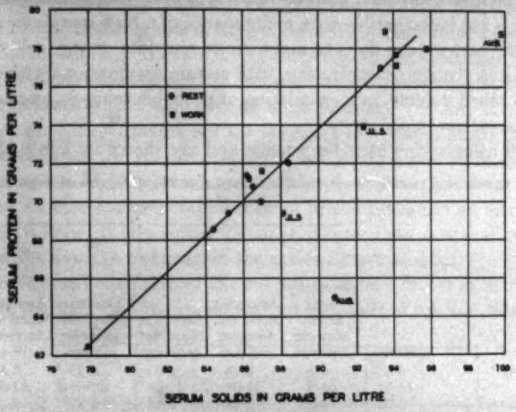

Fig. 1. Relation of serum solids to serum protein in rest and work.

which shows that in most subjects the value for serum solids is merely a linear function of that for serum protein in rest and work. Also it is evident that increase in serum solids is nearly equal to increase in serum protein. Evidently in most subjects concentration of serum fat and other similar substances does not change much in exercise of this character.

The results speak for themselves. It is obvious that in normal man increase in p.c. cell volume during exercise of the sort studied is accompanied by a corresponding increase in serum protein concentration, and the phenomenon is not different in men after splenectomy. Thus the average increase in normal men was 5·6 p.c. and after splenectomy 4 p.c. Clearly the spleen is not essential to this aspect of the phenomenon and seems to play a subordinate rôle under the circumstances of our experiments[1].

[1] Posture effect upon composition of the blood has been studied by W. O. Thompson, P. K. Thompson and M. E. Dailey, *J. Clin. Inv.* 5. 573 (1928). They found changes in

The question as to what changes may have taken place in total blood volume during the course of work cannot be answered. Broun's results(28) on the dog cannot be applied without reservation to our experiments, both because of the difference in species, and because in his experiments at least 4 minutes elapsed after cessation of exercise before blood samples were withdrawn. The marching experiments of Chiatellino and Margaria(31) were of long duration and direct comparison cannot be made, but their findings make it appear that decrease in blood volume is not incompatible with maintenance of a high metabolic rate in man. The best we can do is to make use of our data on p.c. cell volume and plasma protein concentration, with certain assumptions with regard to total blood volume, in a calculation of probable range in amount and character of new blood.

Such calculations have been made and are shown in Table IX. On

TABLE IX. Outcome of certain assumptions with regard to blood volume changes in exercise*.

		Work					
	Rest	Assuming total serum proteins constant	Assuming blood vol. decrease =5 p.c.	Assuming blood vol. constant	Assuming blood vol. increase =5 p.c.	Assuming blood vol. increase =10 p.c.	Assuming blood vol. increase =5 p.c. and cell vol. of new blood =70 p.c.
Blood vol. (litres)	5·0	4·7	4·75	5·0	5·25	5·50	5·25
Cell vol. (p.c.)	44·5†	46·8	47·0†	47·0†	47·0†	47·0†	48·0
Total cell vol. (litres)	2·23	2·20	2·23	2·35	2·47	2·59	2·52
Total serum vol. (litres)	2·77	2·50	2·52	2·65	2·78	2·91	2·73
Serum protein (g. per litre serum)	68·6†	76·0†	76·0†	76·0†	76·0†	76·0†	76·0†
Total serum protein (g.)	190	190	192	201	211	221	208
New blood (litres)	—	None	0·05	0·30	0·55	0·80	0·55
New cells (litres)	—	None	0·03	0·15	0·28	0·40	0·34

* Serum protein concentration in new blood is assumed to be the same as the observed value in our blood specimens. Serum protein concentration change indicates a transfer to tissues of 0·3 litre of salt solution and this transfer is implicit in all our assumptions. Cell volume of new blood is assumed to be 50 p.c. in every case but the last.

† Observed value.

the assumption that total serum protein and total hæmoglobin in the circulation remain constant, while in fact serum protein concentration increases from 68·6 to 76·0 g. per litre, it follows that blood volume decreases 6 p.c. Now in order to restore the original volume, 0·3 litre of blood must be added, and in order to increase p.c. cell volume from 46·6

serum protein concentration and in p.c. cell volume from a reclining to an erect posture, with a minimum of muscular movement, of the same order of magnitude as we have found in exercise. Total serum volume decreased in a reciprocal manner with serum protein concentration and total cell volume remained constant.

to 47·0 the added blood must contain 52 p.c. cells. When blood volume increases 5 p.c., 0·55 litre of new blood is added. The fact that observed p.c. cell volume exceeds calculated cell volume by only 0·2 p.c. thus implies that added blood contains only about 5 p.c. more cells than circulating blood. The experimental evidence on this point is, however, quite inconclusive in any case and wholly without meaning if the volume of added blood is small.

It was found by Bock, van Caulært, Dill, Fölling and Hurxthal(21) that increase in oxygen capacity is a function of metabolic rate. The present experiments involve increases in metabolic rate of eight to twelve times the resting level. In other running experiments at faster rates, increases in p.c. cell volume are sometimes, but not invariably, greater. Such experiments are for the most part unpublished, but one may be referred to. Hochrein, Dill and Henderson(32) found in an experiment on M. H., running at a rate of 11·3 km. per hour for 15 minutes, an increase in metabolic rate of fifteen times and an increase in p.c. cell volume of one-ninth, a change greater than any and twice as large as the average observed in the present experiments.

The physical condition of the subject may be a factor in increase in p.c. cell volume. In our experiments no well-defined relation was found

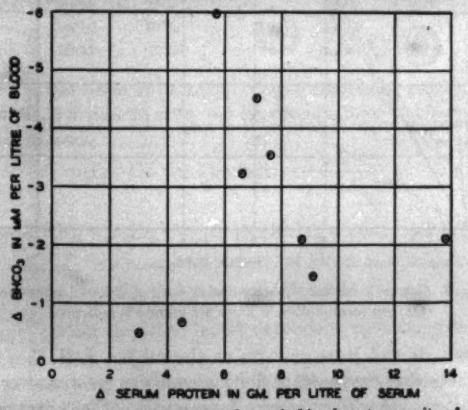

Fig. 2. Serum protein change in relation to change in bicarbonate capacity of whole blood.

between decrease in level of the carbon dioxide dissociation curves and serum protein increments (see Fig. 2). Since the first variable furnishes an

index to physical condition and the second to increase in p.c. cell volume, no relation between physical condition and increase in p.c. cell volume is revealed by the present experiments. Furthermore Bock, van Caulært, Dill, Fölling, and Hurxthal(21) found about the same increase in oxygen capacity in De Mar, a successful Marathon runner for 20 years, as in untrained subjects.

The time factor must also be considered. The above-mentioned authors found in one experiment that while most of the increase in oxygen capacity had taken place at the end of 6 minutes, there was a further increase of about 0·5 volume p.c. between 6 and 16 minutes after work began. In long-continued work concentration of cells in blood may be appreciably influenced by water loss from the body as a whole or by increased rate of destruction of cells or by both factors combined. In Fig. 3 are plotted results obtained by Talbott, Fölling, Henderson,

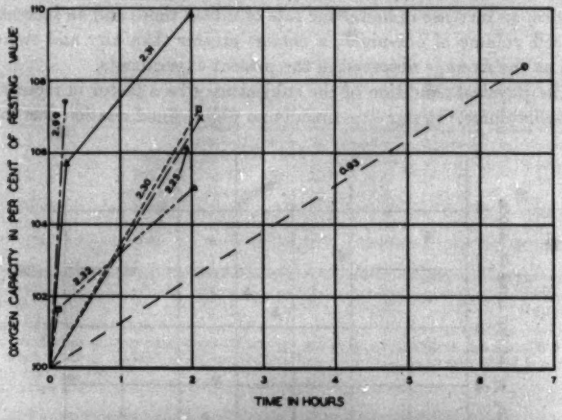

Fig. 3. Change in oxygen capacity during work of different intensities. Oxygen consumption in litres per minute is indicated.

Dill, Edwards and Berggren(33) on the subject J. H. T. It is interesting to note that oxygen capacity increase was greater after a 6-hour walking experiment with an oxygen consumption of 0·93 litre per minute than after three out of four running experiments lasting about 2 hours with an oxygen consumption of about 2·3 litres per minute. J. H. T. drank no water during these experiments so that appreciable cell de-

struction may have taken place while its effect may have been masked by body water loss.

Concentration changes in hæmoglobin of cells. In the above discussion the terms oxygen capacity and cell volume have been used interchangeably, thus implying a constant ratio between these variables in rest and work. The fact that the average increase in cell solids from rest to work is less than 1 p.c., as shown in Table VIII, implies that ratio of oxygen capacity to cell volume does not change notably. Further evidence that such is the case is found in Table X. Hæmoglobin concentrations are

TABLE X. Hæmoglobin concentration at $p\mathrm{H}_s = 7{\cdot}4$.

Subject	Rest (mEq. per l. cells)	Work (mEq. per l. cells)	Δ	Rest (mEq. per kg. cell solids)	Work (mEq. per kg. cell solids)	Δ
D. B. D.	19·84	19·95	+0·11	53·78	53·36	−0·42
P. F. P.	19·48	20·08	+0·60	52·70	53·65	+0·95
A. A. McC.	18·14	19·00	+0·86	48·38	50·26	+1·88
J. H. T.	20·26	20·67	+0·41	53·52	54·30	+0·78
W. C.	20·61	20·42	−0·19	54·20	53·76	−0·44
O. S. L.	20·70	20·69	−0·01	53·42	53·73	+0·31
J. L. S.	20·31	20·43	+0·12	53·94	53·98	+0·04
H. T. E.	20·27	20·28	+0·01	54·20	53·61	−0·59
A. V. B.	20·25	20·37	+0·12	53·95	52·28	−1·67
W. J. G.	21·47	22·25	+0·78	55·26	56·78	+1·52
Average ...	20·13	20·41	+0·28	53·34	53·57	+0·24
Minimum ...	18·14	19·00	−0·19	48·38	50·26	−1·67
Maximum...	21·47	22·25	+0·86	55·26	56·78	+1·88

calculated to the basis of (*a*) mEq. oxygen combining capacity per litre of cells at $p\mathrm{H}_s$ 7·4, and (*b*) mEq. per kg. of cell solids. The first calculation is made as follows:

$$\text{mEq. Hb per litre cells} = \frac{(\text{total Hb})_b}{V_c - \frac{7{\cdot}40 - p\mathrm{H}_s}{30}},$$

where $(\text{total Hb})_b$ = mEq. oxygen combining capacity per litre of blood,

V_c = c.c. of cells per c.c. of blood as equilibrated,

$p\mathrm{H}_s$ = pH of serum as equilibrated,

and $\frac{7{\cdot}40 - p\mathrm{H}_s}{30}$ is an empirical factor dependent upon the rate of change of cell volume with $p\mathrm{H}_s$. The correction in no case exceeds 0·003 in V_c and is thus nearly but not quite negligible. The second calculation is made by dividing the values for oxygen combining capacity per litre of cells, Table X, by corresponding values for solids per litre of cells given in Table VIII.

The first three columns in Table X suggest that the slight increase in

cell solids from rest to work shown in Table VIII is a reality, for there is a similar increase in oxygen combining capacity. The increase in the first case is 0·8 p.c. and in the other, 1·4 p.c.

The values for Δ in the last column of Table X probably can be taken as a measure of the accuracy of the six determinations involved in this calculation, viz. oxygen capacity, cell solids, and cell volume both in rest and work.

Aside from the evidence that rest to work change in hæmoglobin concentration per litre of cells is small and that change in the ratio hæmoglobin ÷ cell solids is probably negligible, Table X is interesting in showing the normal range of individual variation. Values for hæmoglobin concentration per litre of cells are already on record for four individuals of this group (34). These observations were made a year earlier by the same method, and it is of interest to compare the results, shown in Table XI.

TABLE XI. Cellular hæmoglobin concentration in rest. Concentrations in mEq. per litre of cells.

Subject	1928	1929
D. B. D.	19·7	19·8
J. H. T.	20·4	20·2
H. T. E.	21·0	20·2
A. V. B.	20·2	20·2

In three cases the results are practically identical. Only in one case is the difference nearly, if not quite, outside the probable limit of error, and in this case evidence exists that the condition of the subject changed during the period in question. Certainly concentration of hæmoglobin in cells is a variable that fluctuates within narrow limits in normal man, whether at rest or at work.

Acid-base equilibrium in serum. Concentrations of individual anions and of Σ cations in true plasma at a carbonic acid pressure of 40 mm. Hg are shown in Table VI. Values for total CO_2, serum volume, chloride, and phosphate were determined directly and, where the equilibration CO_2 pressure differed appreciably from 40 mm., correction was made to this value. Phosphate corrections are not required and all others can be made by reference to a nomogram of blood of normal man in rest and work, *e.g.* that of M. H. (32).

Values for *p*H are calculated from the equation

$$pH_s = pK_s + \log (BHCO_3)_s - \log (H_2CO_3)_s,$$

where

$$pK_s = 6{\cdot}10,$$

$$\text{mEq. } (H_2CO_3)_s = 0{\cdot}0329\ (H_2O)_s\ (pCO_2),$$

and

$$(BHCO_3)_s = (\text{total } CO_2)_s - (H_2CO_3)_s,$$

accepting the recent determinations of Van Slyke, Sendroy, Hastings and Neill(35) for the solubility of CO_2 in plasma at 38° and correcting it to 37·5°, the temperature we have employed for equilibration. The value used for pK_1 is that recently established by Hastings, Sendroy and Van Slyke(36).

Lactate in serum is calculated from its determination in whole blood, observation of serum volume and the distribution ratio between serum and cells reported by Edwards, Hochrein, Dill and Henderson(37).

Proteinate is calculated according to Van Slyke, Hastings, Hiller and Sendroy(38).

$$\text{mEq.}\ (BP)_1 = 0{\cdot}104\ (P)_1\ (p\text{H}_1 - 5{\cdot}08),$$

where $(P)_1$ = g. of protein per litre of serum.

The expression Σ anions represents the sum of these determined and calculated anions, while Σ cations represents the sum of determined values for calcium, sodium, and potassium, as given in Table III, and the value of 2·0 assumed as representing concentration of magnesium in normal serum.

The results are too involved to yield to simple treatment and hence

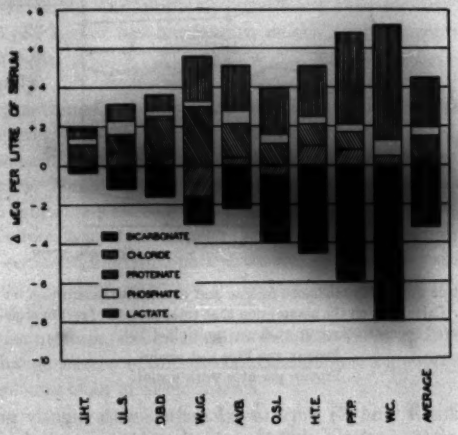

Fig. 4. Increments in principal anions of serum from rest to work.

have been presented graphically in two forms. Fig. 4 shows changes per litre of serum from rest to work. Fig. 5 shows concentration of base and

each principal anion in rest and also the consequences of (*a*) work without exhaustion with lactic acid accumulation small or negligible but with salt

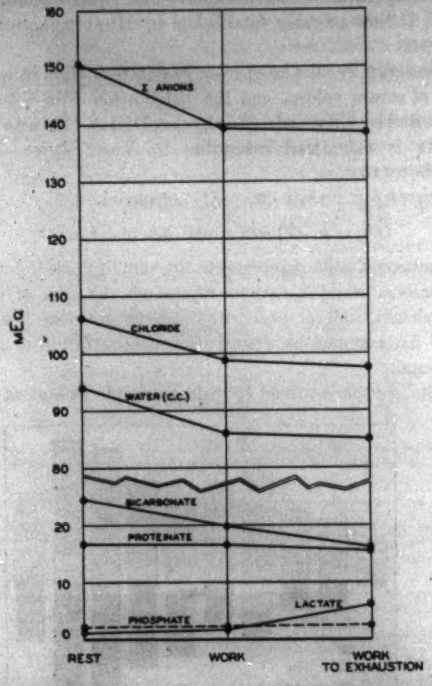

Fig. 5. Effect of work with moderate fatigue and of work to exhaustion on constituents of serum. Calculated on the assumption that total protein of serum remains constant, viz. observed concentrations in work are multiplied by

$$\frac{\text{Protein per litre rest serum}}{\text{Protein per litre work serum}}.$$

solution transfer, and (*b*) work to exhaustion with slightly greater salt solution transfer to tissues and large lactic acid accumulation.

In some particulars, the results are easily understood. Thus there is the reciprocal relation of bicarbonate and lactate already known to occur in whole blood from the work of Bock, van Caulært, Dill, Fölling,

and Hurxthal(21). Also increase in phosphate has already been described by Havard and Reay(39).

On the other hand, there are certain questions heretofore unanswered to which answers can now be given. Thus, on the average, increase in lactate is actually only three-fourths or four-fifths of the decrease in bicarbonate. Taking the subjects individually, Δ lactate as compared with − Δ bicarbonate is distinctly smaller in five cases, larger in one case and about the same in three cases. Chloride shows a slight increase in seven out of nine subjects with an average increase of 0·2 mEq. or less than 0·2 p.c., thus indicating that the fluid leaving the blood during exercise carries a concentration of chloride equal to that of the blood.

Base bound by protein increases on the average just one-half as much as lactate but there are large individual differences. As shown in Fig. 4, there is a fairly uniform increase in serum protein concentration in most of the subjects, even including those most fatigued, P. F. P. and W. C. Consequently when there is little lactic acid accumulation ΔBP_s is large, since ΔP_s increases and (pH_s − 5·08) remains constant or nearly so. But the latter factor decreases with increasing lactic acid accumulation and in the most fatigued subject, W. C., $\Delta BP_s = 0$.

It is interesting and probably significant that for the series $\Delta\Sigma$ anions = $\Delta (BP)_s$, *i.e.* net increase in anions equals increase in non-diffusible anions. Finally, $\Delta\Sigma$ anions = $\Delta\Sigma$ cations from rest to work, approximately.

In short, nothing takes place in the serum which does not have a fairly simple interpretation. In the well-trained subject there is transfer from blood to tissues of a fluid carrying practically unchanged concentrations of diffusible ions. Non-diffusible proteinate with an equivalent amount of base is left behind and there is a higher concentration of phosphate than corresponds to salt solution transfer. This latter change is probably a reflection of increased inorganic phosphate concentration in tissues. In the poorly-trained subject, entrance of lactate decreases bicarbonate and increases acidity.

Values for calcium, sodium, and potassium as given in Table III show no other change worthy of note except that there is a greater relative increase of calcium than of other cations.

Acid-base equilibrium in whole blood. Table VII and Fig. 6 give data for whole blood corresponding to those for true plasma already described. Changes in blood are due not only to changes in plasma and cells but also to change in relative proportion of the two phases. It has already been shown that there is almost no change in concentration of non-diffusible

constituents of cells and it is also true that there is but a slight change in chloride, sodium, and potassium, all of which were determined directly.

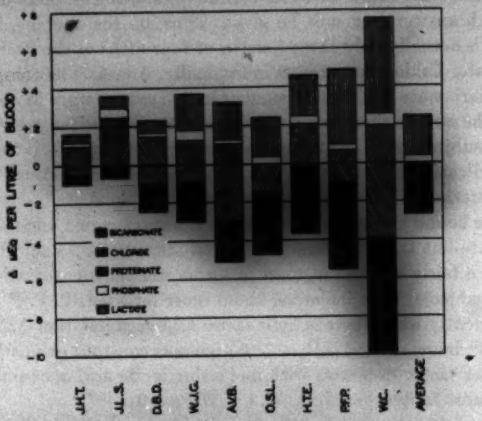

Fig. 6. Increments in principal anions of blood from rest to work.

The components which do change, therefore, are those subject to change with acidosis, viz. $(BP)_c$, lactate, and bicarbonate. The composite picture of blood happens to be simple when the average of the group is considered. Chloride and proteinate are unchanged, ratio of lactate change to bicarbonate change is essentially the same as in plasma, and $\Delta\Sigma$ cations = $\Delta\Sigma$ anions = 0, approximately.

It must be emphasized, however, that blood changes in non-fatigued subjects vary in many respects from those of nearly exhausted subjects. The manner in which $\Delta\,(BP)_b$ changes with acidosis is shown in Fig. 7. When acidosis is no more than moderate there is an increase in $(BP)_b$ of about 1 mEq. The reason for this is that there is, per litre of blood, not much change in serum protein but a large increase in cell protein. With increasing acidosis there is little if any further increase in blood protein but decreasing amount of base found by protein.

The data given in the above tables may be used for calculating concentration ratios of bicarbonate and chloride ions in cells and plasma. A series of preliminary experiments has already been reported by Edwards, Hochrein, Dill and Henderson(37). Their findings have been confirmed in the present experiments, as shown in Table XII.

TABLE XII. Average values for r_{Cl} and r_{HCO_3} at $pH_s = 7{\cdot}45$.

Remarks	r_{Cl} Rest	r_{Cl} Work	r_{Cl} Δ	r_{HCO_3} Rest	r_{HCO_3} Work	r_{HCO_3} Δ
12 subjects, 1928	0·640	0·655	+0·015	0·743	0·784	+0·041
10 subjects, 1929	0·633	0·645	+0·012	0·757	0·768	+0·011
2 subjects with maximum fatigue, 1929	0·655	0·665	+0·010	0·775	0·815	+0·040

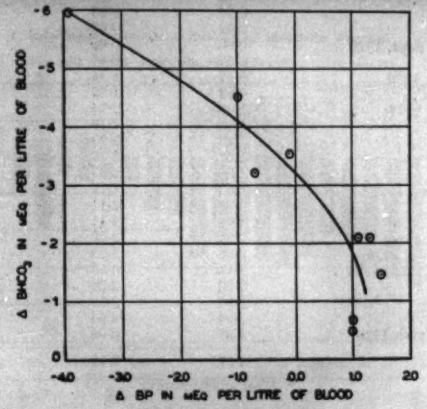

Fig. 7. Base bound by blood proteins in relation to change in bicarbonate capacity of whole blood.

Further, it appears that r_{HCO_3} increases with acidosis, since the most fatigued subject in 1927 and the two most fatigued subjects in 1928 had the greatest average increase in this variable.

Lactic acid accumulation and physical performance. The relation of lactate to bicarbonate change in exercise of this character has been observed on a total of 19 individuals with two or more experiments on five individuals. All these observations are collected in Table XIII and shown graphically in Fig. 8. Subjects are arranged in order of increasing lactic acid concentration, and it will be noted that this is approximately the order of decreasing bicarbonate concentration. This type of experiment furnishes an excellent guide to capacity of individuals for great and sustained physical activity. De Mar, with no appreciable change in lactic acid, is a Marathon runner of 20 years' experience. T. K. was a first-class half-miler 4 years ago and has kept in good condition since then. Probably neither of these men was more fatigued by exercise of this nature than an average person would be by a 20-minute walk.

TABLE XIII. Lactate and bicarbonate changes from rest to work. Concentrations are expressed in mEq. per litre of blood at $pCO_2 = 40$ mm.

Subject	Lactate increase	Bicarbonate decrease	Bicarbonate minus lactate
C. De M.	0·01	0·00	−0·01
T. K.	0·24	0·00	−0·24
J. H. T., March 1929	0·43	0·49	+0·06
D. B. D., 1928	0·44	1·93	+1·49
W. K.*	0·50	0·45	−0·05
B. F. J.	0·63	0·63	0·00
J. H. T., Sept. 1929	0·66	0·60	−0·06
J. L. S.	0·67	0·67	0·00
D. B. D., 1929	0·67	1·44	+0·77
A. A. McC.	0·93	1·62	+0·69
H. T. E., 1928	1·25	2·74	+1·49
G. D.	1·40	2·16	+0·76
E. H.*	1·49	2·20	+0·71
C. H.	1·67	2·70	+1·03
A. V. B., 1928	1·68	2·97	+1·29
W. C., Oct. 1929	1·68	2·72	+1·04
W. J. G.	1·92	2·11	+0·19
A. V. B., 1929	1·97	2·11	+0·14
H. T. E., Sept. 1929	1·97	2·20	+0·23
O. S. L.	2·06	3·23	+1·17
H. T. E., April 1929	2·14	3·55	+1·41
H. W.	2·20	2·34	+0·14
E. F. W.	3·84	3·33	−0·51
P. F. P.	3·94	4·54	+0·60
W. C., March 1929	4·97	5·98	+1·01
Average...	1·57	2·11	+0·54

* After splenectomy.

At the other end of the series stands W. C., a young man of 18. A twentieth subject had to quit from exhaustion in 6 minutes. His record is not shown in the figure, for his lactic acid determination was lost, but bicarbonate decrease was 5·2 mEq. per litre. The fact that there is such a wide range in performance is all the more interesting when one bears in mind that these individuals would be rated as normal by an examining physician. It is another illustration of the old principle that the performance of a machine can be judged best, not when idling, but when running under a heavy load.

One variable in performance can be easily evaluated. It is well known that skill in performance of any physical task varies widely. Running calls for skill of a high order, and it has already been shown by Furusawa, Hill, Long and Lupton(1) that oxygen consumption of different individuals varies considerably while running at a given rate. In Fig. 9 comparison has been made of skill in running of 17 individuals. Variation of net oxygen consumption per kg. of body weight ranges from 26 to 40 c.c., the least skilful subject using one-half more fuel than the most skilful. The data upon which this figure is based together with physical

measurements of some of the subjects are given in Table XIV. While there is a fair correlation between skill and lactic acid accumulation,

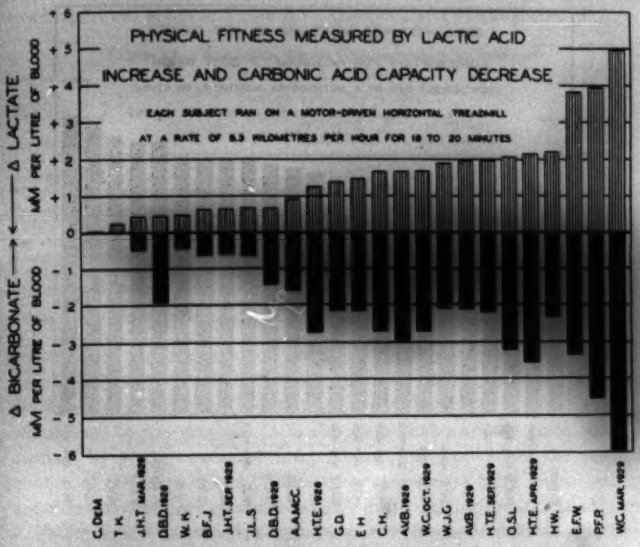

Fig. 8. Increments in blood lactate and in bicarbonate capacity at 40 mm. Hg from rest to work.

TABLE XIV. Physical characteristics and skill index.

Subject	Age (years)	Height (cm.)	Weight (kg.)	Vital capacity standing (c.c.)	Net oxygen used in work (l. per min.)	Skill index
D. B. D.	37	180	72·5	4400	1·91	26·3
P. F. P.	33	173·5	67·6	4550	2·19	32·3
A. A. McC.	23	177·0	74·0	5900	2·70	36·5
J. H. T.	26	171·0	70·4	4000	1·85	26·3
W. C.	18	171·0	62·0	4500	2·49	40·2
O. S. L.	27	188·0	67·2	5950	2·31	34·3
J. L. S.	41	183·2	65·3	5400	2·20	33·7
H. T. E.	32	166·0	67·7	3940	2·04	30·1
A. V. B.	40	173·4	74·2	4000	2·13	28·7
W. J. G.	26	171·0	63·3	4550	2·11	33·2

A. A. McC. is a striking exception. He is one of the most inexpert of runners and at the same time one of the least fatigued subjects. It is not

improbable that of the entire group he would show greatest improvement in training, for he is already well equipped for oxygen transport.

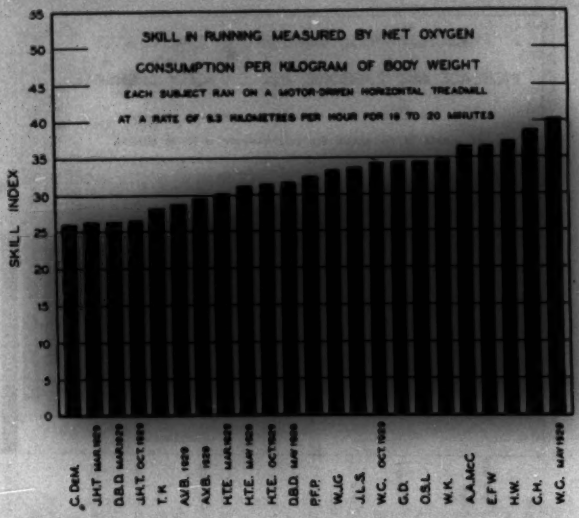

Fig. 9. Skill index expressed as net oxygen consumption per kg. of body weight.

Two or more sets of observations on lactic acid and carbonic acid capacity have been made in the case of five individuals performing the same task at different times. J. H. T., D. B. D., and A. V. B. had about the same lactic acid concentration in 1929 as in 1928. H. T. E. showed only small variation while W. C. in his second experiment showed great improvement. W. C. was the only one of these subjects to show much change in oxygen consumption, using in the second experiment about 2·4 litres of oxygen per minute as compared with 2·8 in the first experiment.

Total ventilation. It is possible from these experiments to study ventilation in relation to time, fatigue, and reaction of arterial blood. The ten subjects may be divided into three groups on the basis of degrees of fatigue. Group I, with less than 1 mEq. lactic acid increase, contains J. H. T, J. L. S., D. B. D., and A. A. McC. The second, with increases of about 1 to 2 mEq., includes W. J. G., A. V. B., O. S. L., and H. T. E.

The last group consists of P. F. P. and W. C. with increases of 3·95 and 4·96 mEq. respectively. Average values for surface area, body weight and for the six observations on ventilation throughout the 20-minute experiment are given in Table XV. Groups I and II show no significant

TABLE XV. Ventilation during work in relation to time and lactic acid accumulation.

Average values by groups.

	Minimum lactic acid; 4 subjects	Medium lactic acid; 4 subjects	Maximum lactic acid; 2 subjects
Surface area (sq. m.)	1·87	1·87	1·77
Weight (kg.)	70·5	68·1	64·8
Ventilation, 3rd min. (l. per min.)	52·3	45·3	54·0
Ventilation, 5th min. (l. per min.)	53·0	50·3	61·5
Ventilation, 7th min. (l. per min.)	53·3	49·5	61·5
Ventilation, 13th min. (l. per min.)	54·3	52·5	70·5
Ventilation, 15th min. (l. per min.)	52·3	51·8	71·0
Ventilation, 17th min. (l. per min.)	53·0	51·3	72·0
Ventilation, average (l. per min.)...	53·0	50·1	65·1

difference except that average weight of the second group is about 3 p.c. less, and average ventilation is about 5 p.c. less. Group III, however, as compared with Group I has an average weight 8 p.c. less and an average ventilation 34 p.c. greater. It is also clear that a steady state was not reached by these two most fatigued subjects until after the seventh minute of the experiment. These observations are fully borne out by unpublished running experiments made in this laboratory on the subjects J. H. T., M. H., and D. B. D. As long as little lactic acid accumulates ventilation reaches a steady state within 3 or 4 minutes. When work is increased enough to cause large lactic acid accumulation, there is a disproportionate increase in ventilation, and instead of early attainment of a constant value the rate continues to accelerate, either until a steady state at a high level of lactic acid is reached, or until the subject stops from exhaustion.

The relation between reaction of the blood and increase in ventilation in exercise is a familiar subject of discussion. Values for pH, in rest and work[1] have already been given in Table IV. In Fig. 10 we have shown pH of arterial serum in rest and at the end of work as a function of total ventilation. Fig. 11 shows pH, as a function of change in ventilation

[1] A systematic error is involved in these calculations of pH, in rest and work for the assumption of a temperature of 37·5° is made. Temperature in rest is somewhat below this and in work of this intensity possibly somewhat higher. Since we did not observe body temperature in these experiments no attempt has been made to correct for the small error involved in the assumption of a constant body temperature.

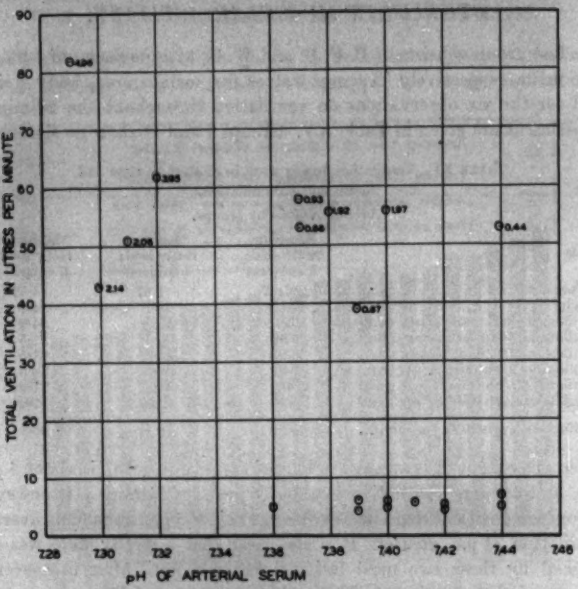

Fig. 10. *p*H of arterial serum as a function of total ventilation in rest and work. Increase in lactic acid concentration in mEq. per litre of blood is indicated.

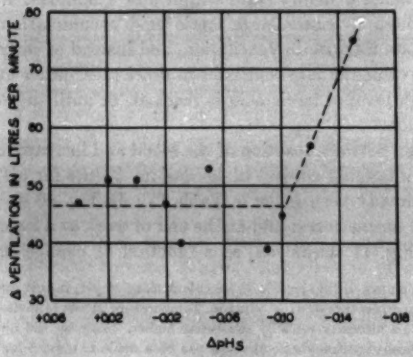

Fig. 11. Change in *p*H$_s$ as a function of change in ventilation from rest to work.

from rest to work. It is clear that individual variation, both in rest and work, is considerable and that many subjects have a reaction in work well within normal limits for rest. Values in Fig. 10 indicate blood lactate concentration and illustrate again the relation between ventilation increase and lactate concentration. The two subjects with greatest lactate concentration had also greatest ventilation increase.

Increase in ventilation is of course a function of body weight. This latter variable has been eliminated in Table IV which gives values for pH, and ventilation per kg. body weight. Here again there are large individual variations. Nevertheless it is quite clear that the relation between decrease in pH, and increase in ventilation is not linear. As has been pointed out by L. J. Henderson(12), it is clearly impossible to select one variable, such as the hydrogen-ion concentration of arterial blood, and assign to it a unique influence upon the respiratory centre.

Respiratory quotient. This is not a suitable occasion for reviewing the extensive literature upon the R.Q. in exercise, particularly in view of the recent review by Richardson(40). Values have been reported ranging from well below the usual resting value to unity and above. For example, there are the experiments on college oarsmen of Henderson and Haggard(41) which led them to the conclusion that fat and sugar are burned in the same ratio in rest and work, provided one takes into account the recovery process. The results of Hill and his associates(1) point to an R.Q. of unity for severe exercise of short duration plus recovery. In more moderate exercise involving small oxygen debt their R.Q.s are well below unity. Experiments of particular interest from our point of view are those of Benedict and Cathcart(42), who carried out a long series of bicycle ergometer experiments on M. A. M., a professional cyclist. We have collected their values for M.R. and R.Q. in exercise from their Tables Nos. 36–52, 59–62, 68, and 70–90, not including experiments with special diets. Results for each table were averaged except where it was possible to group observations into two or more distinct metabolic levels. Sixty-eight pairs of such observations are shown in Fig. 12. The best fitting straight line for these points[1] corresponds to the equation

[1] The straight line shown on Fig. 12 was derived according to the formula

$$y = Rx - Rx_m + y_m,$$

where x_m = mean value of x,

y_m = mean value of y,

n = number of observations,

and $$R = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}.$$

R.Q. = 0·04 (M.R.) + 0·817, where M.R. is expressed in litres of oxygen used per minute. The results leave no doubt that in these experiments

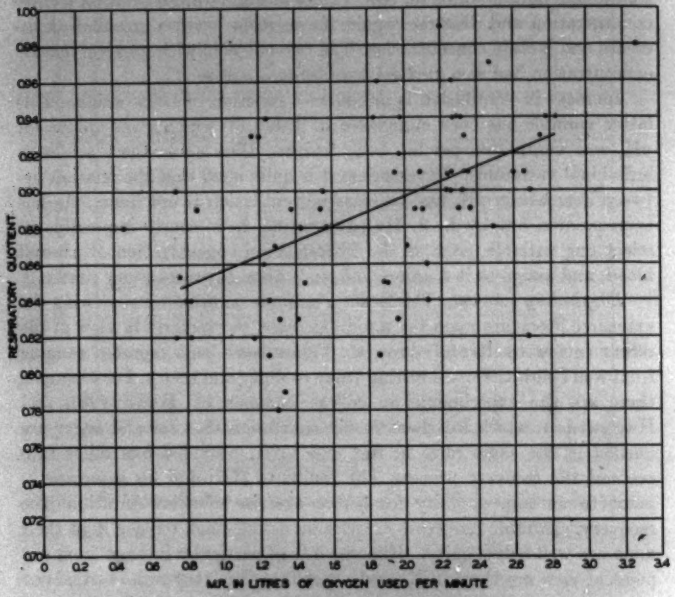

Fig. 12. Change in R.Q. with M.R. Calculated from Benedict and Cathcart(42).

there was a well-defined increase in R.Q. with M.R. This was noted by Benedict and Cathcart(42), who remarked:

"Apparently in the later experiments there was an increase in the respiratory quotients during the working period which was not observed in the earlier experiments, this probably being due to the fact that the excessive muscular work was performed in the last part of the research."

Bock, van Caulært, Dill, Fölling and Hurxthal(21) also found values increasing with metabolic rate. De Mar, a trained athlete, had the lowest values of the group of four men studied. Talbott, Fölling, Henderson, Dill, Edwards and Berggren(33) in 2-hour experiments on J. H. T. found R.Q.s of about 0·88 at a metabolic rate of about 2·3 litres of oxygen per minute. Unpublished observations on J. H. T.

using about 2·3 litres of oxygen per minute on the bicycle ergometer give values for R.Q. above 0·9 and similar observations have been made on other individuals.

The values given for respiratory quotient in rest in Table II range from 0·72 to 0·88 and average 0·80. Recent observations of Benedict (43) on variation in the R.Q. in rest when a mouthpiece is employed, and its constancy when a suitable mask is used, leave little doubt that our three quotients in rest which depart widely from the average value give a misleading impression of the resting metabolic processes going on in those subjects.

Our evidence, however, that the R.Q.s reported for work indicate approximately what ratio of fat and carbohydrate is being used is as follows:

Carbon dioxide is being produced at about ten times the resting rate. Since the total carbonic acid content of the body is of the same order of magnitude in rest and work, ten times as much would have to be blown off or held back in a given time in order to produce a given change in R.Q. Our observations on alveolar air and on the level of the carbonic acid dissociation curve in rest and work tell us to what extent such a blowing off or retention of carbonic acid has taken place. On the average the change in alveolar carbonic acid pressure is small, as has been pointed out already. Accordingly we have again segregated the ten subjects into groups according to lactic acid accumulation. The R.Q.s thus summarized are presented in Table XVI.

TABLE XVI. Respiratory quotients during work in relation to time and lactic acid accumulation.

Average values by groups.

	Minimum lactic acid; 4 subjects	Medium lactic acid; 4 subjects	Maximum lactic acid; 2 subjects
3rd min.	0·90	0·90	1·00
5th min.	0·92	0·94	1·03
7th min.	0·91	0·92	0·98
13th min.	0·90	0·92	1·00
15th min.	0·89	0·92	1·00
17th min.	0·90	0·89	0·98
Average...	0·903	0·915	1·00

Groups I and II, with little or moderate lactic acid concentration, and hence small blowing off of CO_2, again show only slight difference in values for R.Q., the averages being 0·903 and 0·915, respectively. There does appear to be a slight falling off in R.Q. as work continues. Group III,

with maximum fatigue, has quotients of unity. P. F. P. showed a distinct falling off towards the close of the experiment, while W. C. evidently continued to blow off carbon dioxide to the end. We are inclined to accept the quotient 0·91 as a true indication of what materials were being oxidized in Groups I and II. The average M.R. for these groups was 2·41 litres of oxygen per minute and it is interesting that the line indicating the trend of the experiments of Benedict and Cathcart passes through the point (M.R. = 2·41, R.Q. = 0·91). Not much can be said as to fuels used by the third group. A more satisfactory procedure when exhaustion and blowing off of CO_2 occur is that of Hill, Long and Lupton(44), who followed oxygen consumption and carbon dioxide production through work and complete recovery. Such experiments gave quotients of about unity. Even in such experiments there is the possibility that the recovery process may involve a different proportion of fuels than that of work.

Certain facts seem to be well established and are worthy of emphasis:

(*a*) Exercise requiring no more than 1·5 litres of oxygen per minute is often carried on with little or no change in R.Q. The extensive experiments of Benedict and Cathcart(42) prove this.

(*b*) Respiratory quotient increases with metabolic rate. This is proved by the work of Benedict and Cathcart(42), and again by Bock and his associates.

(*c*) It is possible to carry on long-continued experiments on the ergometer at a metabolic rate of about 2 litres of oxygen per minute, with high but constant lactic acid and no blowing off of CO_2. Under such conditions the R.Q. may average 0·94 with a net R.Q. for work of 0·96. This was shown by Henderson, Dill, van Caulært, Fölling and Coolidge(45).

(*d*) In view of the increase in R.Q. with M.R. clearly established by the work of Benedict and Cathcart, our results do not conflict with those of Krogh and Lindhard(46), for their oxygen consumptions were below 1·5 litres per minute.

Pulse and respiratory rates. These observations are particularly interesting in showing to what extent these rates vary among individuals. Respiratory rate in work ranged from 16 in the case of O. S. L. to 44 in the case of W. C. There is a closer relation between pulse rate and performance. Athletes have notoriously slow pulse rates as compared with non-athletes at the same metabolic level. To what extent their increased stroke volume is due to training is quite unknown.

Oxygen and carbon dioxide transport. The composition of alveolar air and of "virtual venous" air as defined by Henderson and Prince(3),

with derived calculations, are shown in Table II. Individual determinations are given for alveolar air. Four samples were collected throughout the experiment, and in general the range from rest to work and during the course of work is not very great. This has already been referred to in Table V, and the relative constancy of alveolar carbon pressure has been used as a basis for recalculation to a common basis for all.

Alveolar oxygen pressure in work fluctuated from 87 to 116 mm. although most values were between 100 and 112 mm. Oxygen saturation of arterial blood was probably about 95 p.c. except in the cases of P. F. P. and W. C. where, on account of lactic acid accumulation and shift of the oxygen dissociation curve, it may have been as low as 92 p.c. Our basis for this conclusion consists of the arterial punctures in exercise reported by Dill, Laurence, Hurxthal and Bock(13), supplemented by two unpublished experiments on the ergometer with lactic acid accumulation somewhat greater than that observed in the cases of P. F. P. and W. C. and with arterial saturations of 91·2 and 91·4 p.c. and with oxygen pressure heads of 35 and 15 mm. respectively.

With these assumptions as to saturation of arterial blood, with the established rate of carbon dioxide production and the calculated values for carbon dioxide and oxygen transport, we have calculated values shown in Table II for venous saturation and rate of blood flow. The eight subjects with least fatigue had venous saturations ranging from 41 to 50, average 44 p.c., while P. F. P. had a value of 39 and W. C. 34. In general those who had higher saturations of venous blood in rest had high values in work also. Differences between venous saturation in rest and work ranged from 31 to 38, and averaged 35 p.c. for all subjects.

Values for venous saturation are more interesting than rate of blood flow for many purposes since the variable of body weight is eliminated, and with a knowledge of the position of the oxygen dissociation curve one can derive the oxygen pressure in blood leaving tissue capillaries. By the methods described by Dill, Talbott, Edwards and Oberg(10), we have estimated pH_e values for mixed venous blood in rest and work. The effect on position of oxygen dissociation curves of changes in pH_e, due to lactic and carbonic acids, has been found to be linear near the physiological range(47), and hence it is simple to interpolate on a family of oxygen dissociation curves and to estimate value for oxygen pressure in each case. The resulting estimates are shown in Table XVII.

The results in work may be considered a striking confirmation of the estimates of L. J. Henderson(12) for four subjects near maximum work

TABLE XVII. Pressure of oxygen in mixed venous blood.

Subject	Rest (mm. Hg)	Work (mm. Hg)	Δ (mm. Hg)
D. B. D.	41	25	-16
P. F. P.	40	26	-14
A. A. McC.	38	25	-13
J. H. T.	46	27	-19
W. C.	39	26	-13
O. S. L.	45	30	-15
J. L. S.	45	27	-18
H. T. E.	44	27	-17
A. V. B.	42	27	-15
W. J. G.	49	27	-22
Average...	43	27	-16

on the ergometer. He has fully discussed the significance of this observation, and has estimated that blood leaving the capillary of an active muscle contains oxygen at a nearly constant partial pressure in the neighbourhood of 20 mm.

SUMMARY.

A study has been made of simultaneous changes in several important physiological variables during performance of a fixed task by ten individuals. Supplementary observations have been made on nine other subjects, including two young men after splenectomy. The task consisted in running at 9·3 km. per hour for 20 minutes with an increase in metabolism of eight to twelve times the resting rate.

Concentration of the blood in exercise by transfer of salt solution to the tissues does not commonly exceed 10 p.c. in man and in these experiments averaged about 6 p.c. in normal man and 4 p.c. in two splenectomized subjects. Changes in serum protein concentration corresponded to changes in red cell concentration. The possibility that the spleen may function in such exercise by increasing blood volume is not involved in these observations and deductions. There is no well-defined connection between physical condition and blood concentration change in exercise.

Concentration of hæmoglobin in cells is remarkably constant in normal man and increases only about 1 p.c. in work of this character.

The effect of exercise on concentration of the principal ions in serum and in cells is as follows:

(*a*) In non-fatigued subjects changes consist chiefly of those related to transfer of fluid carrying diffusible ions from blood to tissues, the exception being a disproportionate increase in inorganic phosphate and calcium of serum.

(*b*) In exhaustion there is but little greater transfer of fluid from blood to tissues, and consequently only slightly greater increase in serum protein

and in blood hæmoglobin concentrations. Lactate increase in blood is approximately balanced by decrease in other ions, notably bicarbonate and proteinate.

(*c*) In general, there is an increase of about 2 mEq. of anions and cations per litre of serum but, on account of the decreased proportion of serum in blood during work, there is on the average practically no change in anions and cations per litre of blood.

The principle that a machine can be judged best when running under a heavy load has been exemplified by comparing blood lactate and bicarbonate changes from rest to work. Subjects who, at rest, would be placed in the same category were differentiated precisely by our observations.

Total ventilation in work of this character reaches a steady state in 3 or 4 minutes if lactic acid is not accumulating rapidly. Even when the lactic acid increase is 4 or 5 mEq. per litre, ventilation rate may become nearly constant in from 7 to 15 minutes. The level reached in such a case may be one-third higher than in non-fatigued subjects. In these experiments there was no evidence of a linear relation between decrease in pH_e and increase in ventilation. When lactic acid does not accumulate, the subject may maintain the resting reaction in arterial plasma despite an eight or tenfold increase in metabolic rate.

Work of this level, when there is not excessive exhaustion, is associated with a change in fuel from the resting ratio of one-third carbohydrate and one-half fat to a ratio of two-thirds carbohydrate and one-third fat.

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612.745.1

THE ENERGY EXPENDED IN MAINTAINING A MUSCULAR CONTRACTION.

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It is obvious that the energy expended by a muscle increases with the strength of the contraction. There is, however, no definite information regarding the relative amounts of energy required to maintain contractions of varying strengths. This question is of considerable importance in connection with the economy of muscular activity, and is of timely interest because of the recent investigations of Adrian and Bronk(1, 2) regarding the mechanism of grading the strength of a muscular contraction.

By recording the frequency of motor impulses in single nerve fibres and the resulting contraction frequency of single groups of muscle fibres, they showed that there are two ways in which the strength of a reflex or voluntary contraction may be increased. An increased number of neuromuscular units may come into action, and a higher tension is produced in the individual fibres by a higher frequency of motor nerve impulses. Rising from a level as low as 5 or 10 a second in a weak tonic contraction to 80 or 100 in the stronger responses, the resulting contraction of the single muscle fibre changes from a series of discrete pulls to a fused tetanus. Due to the fact that the twitches of the various fibre groups are out of phase the response of the muscle as a whole in a weak contraction is smooth and fused, completely masking the discontinuous character of the unit responses. As the frequency of the twitches increases, the resulting summation produces a higher tension and an increase in tension-time. In a steady contraction the tension-time is of course the product of the tension by the time that it is maintained. In a non-uniform contraction or in a series of twitches it is the area under a tension-time curve

[1] The experimental work reported in this paper was done while the author was a Fellow in Medicine of the National Research Council, U.S.A.

or $\int T\,dt$, where T is the tension and t the time. From the point of view of a maintained contraction it is this quantity that is of importance, and in considering the relative energy expenditure in various types of contractions we will use the ratio of the tension-time to heat production or that of the tension to the rate of heat production as a measure of the economy.

The answer then to the question regarding the relative economy of maintaining contractions of varying strengths lies in a study of the energy expended in developing a given amount of tension-time (*a*) when varying fractions of a muscle are in action, and (*b*) when the muscle is stimulated at various frequencies. No attempt has been made to measure the variation in economy of contractions graded by the former mechanism, due to the uncertainty regarding the distribution of heat between muscle and thermopile in submaximal contractions and the consequent suspicion attaching to such myothermic observations. There is, however, no obvious reason why a mere increase in the number of fibres contracting should alter the ratio of tension produced to heat developed, and it seems safe to say that in so far as a stronger contraction is a result of an increased number of active fibres, the economy of maintaining a contraction is probably independent of the strength.

Previous work of Hartree and Hill(3) gives us a clue as to what we may expect to find regarding the variation in economy of maintaining tension at various frequencies. In the paper referred to they show that the heat production in the early part of a short tetanus is relatively greater than that in the later portions. This observation may be logically interpreted as indicating that it is more expensive to develop a muscular tension than to maintain it. If this be so, one would expect to find that it is less economical to produce a given amount of tension-time by a series of twitches than by a fused tetanus, and consequently that a weak contraction maintained by a low frequency of motor impulses would be relatively less economical than a strong contraction resulting from a higher frequency motor discharge.

This has been tested experimentally by stimulating the sartorius muscle of a frog (*Rana temp.*) for a given period of time at various frequencies, observing the tension-time developed and measuring the heat production during the period of stimulation. The methods employed are essentially those which have been developed by Hill during recent years(4). The excised muscle was mounted on a thermopile, placed in a glass vessel containing Ringer's solution to which was added 10 mg. p.c. of P in the form of sodium phosphate, and the whole ensemble mounted in a fairly constant temperature bath enclosed in a large Dewar flask.

After soaking in the phosphate-Ringer's fluid for about 3 hours as a precaution against loss of irritability, the solution was removed and a slow stream of oxygen passed through the vessel throughout the course of the experiment. Just maximal stimuli of various frequencies were delivered to the muscle from a condenser, which was alternately charged and discharged at the desired frequency. This method was employed after four standard contact breakers used in conjunction with a Harvard induction coil were found to involve the danger of chatter and repetitive stimuli at the higher frequencies. These same faulty contactors, when used for charging and discharging the condenser, of course did not give multiple stimulation even though the contacts chattered. The tendon end of the muscle was connected to an isometric lever, which recorded on a rapidly moving drum turned at a constant speed. The tension-time developed during any period of stimulation was, therefore, obtained by determining the area between this tracing and the base line, the lever being frequently calibrated. The initial heat production was observed by means of the thermopile and either a Downing moving magnet or a Kipp moving coil galvanometer. Due to the fact that the galvanometers had periods of from 3 to 6 seconds and the thermopile had been made slow by coating with a considerable amount of paraffin, the heat production during a 1- or 2-second period of stimulation was taken directly as the ballistic swing of the galvanometer. In certain experiments the duration of stimulation was increased to 5 seconds and the frequency reduced to one every 2 seconds. Even then it was found that the thermopile-galvanometer system could be made to sum by adding sufficient paraffin to the thermopile surface.

Provided the interval between successive stimuli is greater than 1 second there is no evident variation in economy with frequency, as is shown by Fig. 1. A further increase in the rate of stimulation, however, results in a greater economy of tension-time production, even though the successive contractions are in no wise fused. This absence of even partial summation precludes the possibility of explaining the increased economy on these grounds. It is rather to be accounted for on the basis of observations made by Hartree and Hill(6), which showed that with regard to the second of two twitches "the efficiency of the contraction, as measured by T/H, is noticeably increased as the result of a previous shock at a suitable interval." Some altered condition of the muscle resulting from the previous contraction persists and makes more efficient the succeeding twitch—thus giving rise to a true "staircase" effect.

Fig. 2 shows the variation in economy over a wider range of frequencies. In this experiment tension-time was maintained nearly three

times as economically at the higher rates of stimulation. Ten other experiments showed that the economy was increased by factors of from

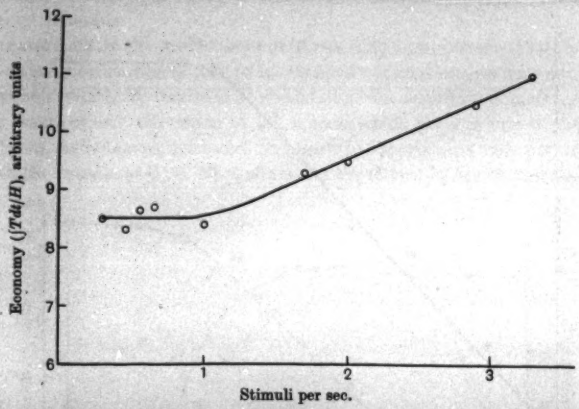

Fig. 1. Variation of economy with low frequencies of excitation. Muscle stimulated for 5-sec. periods. All contractions were discrete twitches with no evidence of summation. 18° C. 17. xii. 28.

three to five. The broken line of Fig. 2 represents the maximum tension developed by the muscle at the various frequencies. At first the tension is independent of the frequency because there is no fusion of the successive twitches; a further increase in the rate of stimulation causes a progressive increase in the degree of fusion, and a consequent increase in tension which continues until the contractions reach the stage of a fused tetanus.

A comparison of these two curves indicates the reasons for the increase in economy with increased frequency of stimulation. The rising economy at low frequencies is to be accounted for on the grounds suggested in the preceding paragraph; the larger part of the increase, however, is to be ascribed to the increased fusion of the successive twitches. This is shown by the fact that the economy and tension both increase with the frequency up to, but not beyond, the limit of complete fusion. In this connection it is interesting to recall that the frequency of motor nerve impulses in reflex contractions does not seem to go above a level adequate to produce maximal tension in the muscle. In view of the fact that the present experiments show the economy and tension to vary over much

the same frequency range, we may now state that as the motor centres speed up their discharge as a result of greater excitation, maximum tension is developed at a maximum economy.

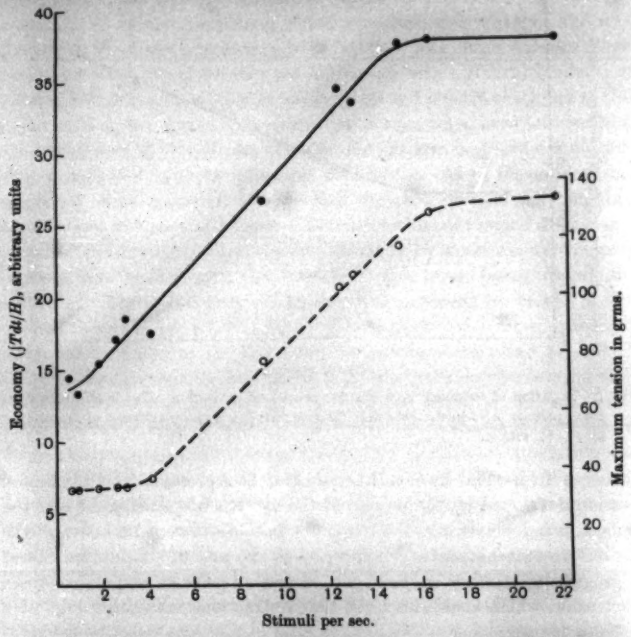

Fig. 2. Solid line: relation between economy and frequency of stimulation. Broken line: relation between maximum tension developed during each 2-sec. period of stimulation and the frequency. 18° C. 12. xii. 28.

It must also be concluded from these experiments that a weak contraction maintained by a series of discrete twitches, or by a partial tetanus of the active muscle fibres, is less economical than if the contraction were the result of complete tetanus of a smaller number of fibres. This lower economy may, however, be offset by the possible advantage of a more adequate circulation in a prolonged contraction sustained by a succession of twitches in the individual fibres. What is more to the point is the fact that the grading of contractions by an increased frequency of

excitation achieves a greater economy as the strength of contraction increases, being a nicely balanced control for lowering the energy required per unit of tension in those contractions which make the greatest total energy demands.

Inasmuch as the greater economy resulting from increased frequency of stimulation is largely due to mechanical summation, we may suppose that at a relatively low rate of stimulation the economy is increased as the result of any alteration in the muscle which gives it slower characteristics. As a demonstration of this, certain experiments were performed with the muscle at 0° C. Typical results are shown in Fig. 3, from which

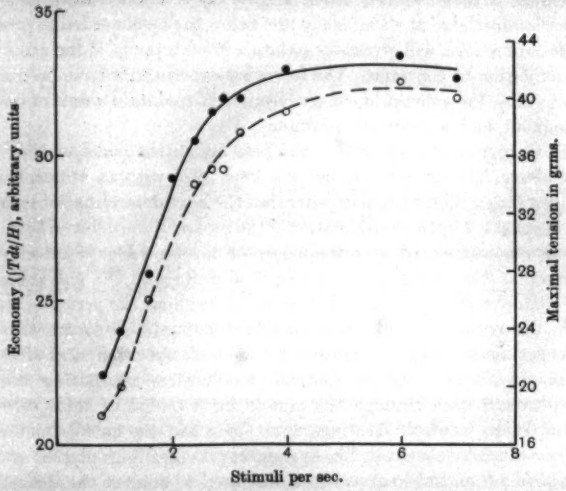

Fig. 3. Muscle at 0° C. Solid line: relation between economy and frequency of stimulation. Broken line: maximum tension developed at the various frequencies. 5-sec. periods of stimulation. 15. xi. 28.

it will be seen that the maximum economy and tension are attained at a stimulus rate of about five a second. The rate of stimulation at which the economy begins to increase is of course also much lower than in the case of a muscle at room temperature. These observations have no obvious bearing on normal reflex and voluntary contractions except in the case of cold-blooded animals. As their body temperature falls, it seems reasonable to assume that the average range of discharge frequency from the

motor centres would decrease, both as a result of lowered temperature of the motor cells themselves (Cooper and Adrian(6)), and as a result of a lower frequency of afferent impulses (Matthews(7)) playing upon these centres. The result of a lower rate of motor impulses would be a contraction maintained at a greater expenditure of energy per unit of tension, were it not for the fact that the lowered temperature of the muscle causes its contractions to fuse at a slower rate and so maintains the higher level of economy.

Effect of fatigue.

An altered muscular condition of more significance in the present discussion is that resulting from fatigue. It is a well-known fact that a muscle stimulated at a frequency just below the level needed to produce partial summation will gradually go into a fused tetanus, if the same rate of stimulation be continued. The following experiments have been made to determine the amount of energy required to maintain a unit of tension throughout such a progressive fatigue.

The experimental procedure has been much the same as that outlined above. The muscle chamber was kept filled with an atmosphere of nitrogen rather than oxygen in order that the heat determinations may be less complicated by the consideration of the recovery and delayed heat. The muscle was stimulated at a frequency of three or four a second, the contraction was recorded on a fairly slow drum and the galvanometer deflection observed at 6-second intervals throughout the period of stimulation. This was continued for several minutes or until the contraction was no longer measurable. The muscle was then electrocuted, and after the system had again reached thermal stability an alternating heating current was passed through the muscle for a period of some seconds. Several such "control" heatings were made and the galvanometer deflections carefully observed. The average of these readings plotted against time gave a "control" curve which was used to analyse the deflection-time curve obtained during the prolonged stimulation of the muscle. Following the methods described by Hartree and Hill(8) this analysis gave the rate of heat production at 6-second intervals throughout the course of the stimulation.

The variation in economy of maintaining tension as a result of previous stimulation is given by dividing the tension-time developed during each of the 6-second intervals by the heat production during the corresponding period. The solid line of Fig. 4 shows that during 2 minutes of continuous stimulation at a frequency of four a second the amount of tension-time produced per unit of energy expended increased more than

fivefold. This increase in economy was characteristic of each of six experiments although the rate and extent of the increase varied somewhat

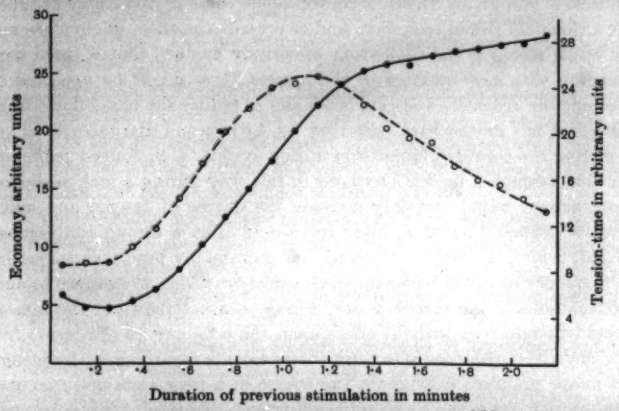

Fig. 4. Solid line: economy of maintaining muscular tension $\frac{\int T dt \text{ in interval}}{H \text{ in interval}}$ with increasing duration of previous stimulation at four a second. Galvanometer deflection analysed at 6-sec. intervals by means of a control heating curve of 5-sec. duration. Broken line: amount of tension-time developed in successive 6-sec. periods. 11. i. 29.

with the previous condition of the muscle. The broken line of Fig. 4 represents the amount of tension-time developed during successive 6-second intervals throughout the course of the stimulation. The rise is obviously due to the gradual increase in summation of the successive twitches as fatigue develops; the subsequent fall results as greater fatigue offsets the gain resulting from fusion. A comparison of these two curves shows that the most rapid increase in economy comes during the period in which the character of the contraction undergoes the most pronounced change from a series of twitches to a fused tetanus. The increase in economy associated with the changed state of the muscle resulting from previous stimulation is, therefore, to be ascribed to the lower energy requirement for maintaining a given tension as compared with that required for the development of a contraction.

Two factors might at first thought indicate the inapplicability of these findings to normal muscular contractions. It may be argued that even in long sustained maximal contractions a circulated muscle would never develop the degree of fatigue produced in these experiments on excised

preparations. It is, however, to be noted that even in the early stages of this fatigue, *i.e.* during the course of the first minute, and while the muscle is still giving vigorous contractions, there is frequently as much as a threefold rise in economy. And as regards the effect of circulation it is worth recalling the suggestion previously made(2) that a fused contraction of a large number of the muscle fibres might be expected to occlude the arterioles and capillaries and so reduce the blood flow. Such an effect has indeed been described by Anrep and Häusler(9) as restricting the coronary circulation during systole. A prolonged reduction in flow through a muscle produced in this way during a long sustained contraction, would certainly increase the degree of fatigue and make possible summation at a lower frequency. But a strong contraction, during which the fatigue develops, has presumably been maintained by a frequency of motor nerve impulses sufficiently high to produce tetanic contractions of the muscle fibres. Slower characteristics of the muscle would therefore be without effect upon the economy at that same frequency. In the absence of experimental evidence regarding the frequency of motor impulses throughout the course of a long sustained maximal contraction, there can be no satisfactory consideration of this point, but it is at least probable that the frequency from the motor nerve cells would gradually decrease as a result of a prolonged high rate of activity. In that event the slower characteristics of the fatigued muscle would more or less effectively compensate for the decreased impulse frequency, checking the fall in tension and economy that would otherwise result.

Certain invertebrate muscles, as for instance the claw muscles of the crab, show a rapid slowing as a result of sustained contraction and their nerves soon fatigue at fairly high rates of stimulation. As Hill(10) has pointed out, this fatigue of the nerves may be prevented by a decrease in the frequency of the motor impulses. Due, however, to its slower characteristics the muscle would still maintain a uniform tension, and without the increase in energy expenditure that would otherwise result from a lower frequency of excitation. Such considerations indicate that the experiments referred to in this paper may give a clue to the puzzling problems regarding muscular contractions which, although rapid in their onset, are nevertheless maintained with an unusually low expenditure of energy. This question is more fully discussed by Dr Bozler in a succeeding paper.

SUMMARY.

The economy of energy expenditure in maintaining muscular contractions has been studied, at various frequencies of stimulation and in progressive fatigue. This has been done by measuring the heat production and the tension-time.

1. As the rate of stimulation is increased, the economy, or amount of tension-time maintained per unit of energy expended, increases. This is largely due to a greater degree of fusion of the separate muscle twitches, there being no further increase in economy with frequencies of a value higher than that needed to produce complete tetanus.

2. These results are discussed in relation to the findings of Adrian and Bronk, which show an increased strength of muscular contraction to be due in part to an increase in frequency of motor excitation. It may, therefore, be concluded that tension is maintained at a smaller expenditure of energy in a strong contraction than in a weak one.

3. At 0° C. the increase in economy develops at lower frequencies. In cold-blooded animals this factor would be of significance in the event that the range of frequency of impulses from the motor centres was lowered as a result of a fall in temperature.

4. A stimulation frequency not quite sufficient to give partial summation of the successive twitches will, if continued, produce a fused tetanus. Associated with this changed condition of the muscle there is a greater economy of maintaining tension. It is pointed out that this factor may play an important rôle in long-sustained contractions where there would be reason for expecting a decreased frequency of motor impulses.

I wish to express my gratitude to Prof. A. V. Hill for his constant advice and for the facilities which have made this work possible. I am also indebted to Mr A. C. Downing and Mr J. L. Parkinson for much technical assistance.

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THE RELATION BETWEEN THE MECHANICAL AND ELECTRICAL RESPONSE OF THE FROG'S HEART.

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Some years ago Mines(1) stated that when the mechanical response of the frog's isolated heart is reduced to less than 1 p.c. of its original strength by perfusion with calcium-free Ringer's fluid, the electrical response remains vigorous, and very often not reduced in strength at all. In one case he obtained a fairly normal electrical response, although no mechanical response was visible.

This work was repeated by Einthoven and Hugenholtz(2, 3), and by Arbeiter(4). They studied the effects of both lack of calcium and excess of potassium on the mechanical and electrical responses of the frog's isolated heart. They concluded that the two kinds of response were reduced *pari passu*, and that the results of Mines were due to technical errors such as inadequate sensitivity of the mechanical recorder. Mines' results were also open to the objection that the deflections of the string galvanometer were not standardized.

Willigen(5) reinvestigated the effect of lack of calcium. He used a delicate recorder and standardized the deflections of his galvanometer. He found that lack of calcium might produce a great reduction of the mechanical response, without any in the height of the initial deflection of the electrical response. This question is of considerable theoretical importance, because all theories of the nature of the electrical response depend upon the view taken of the relation between the electrical and mechanical responses.

Fulton(6) has recently summarized the controversy as follows: "The size of the electrical response varies with the initial length of the muscle fibre in much the same way as heat liberation varies, also the duration of any given mechanical response varies with the size of the electrical response. This again suggests that the mechanism of the reproduction of the electrical response and the mechanism controlling initial heat production are one and the same."

This summary appeared to us to ignore evidence of considerable weight, and in view of the conflicting nature of the evidence we determined to investigate the question.

Procedure.

The electrical response was recorded by means of an Einthoven galvanometer (Cambridge pattern). When the electrical response was measured the string was always standardized. The electrodes consisted of silver wires coated with silver chloride, with cotton wool soaked in Ringer's fluid bound over their tips; when the perfusing fluid was changed the cotton wool was thoroughly soaked with the changed fluid.

"Speedy" (special rapid) plates were used, as it was found that when using plates of 100 H. and D., the true height of the initial deflection was not recorded sharply enough, or failed to show more than the first part of the initial deflection.

These experiments were carried out on the hearts of *R. temp.* from the months of January to May. *R. esc.* were used for the auricular preparations. The Ringer's fluid used had the percentage composition of NaCl 0·65; KCl 0·015; $CaCl_2$ (anhyd.) 0·011; Na_2HPO_4 and NaH_2PO_4 0·04. The phosphate was added from a stock solution with a *p*H of 7·5.

The following preparations were used:

(1) *Whole ventricle.* A two-way cannula was inserted through the auricle into the ventricle, and the ventricle ligatured around the A.–V. groove. A 0·2 c.c. pipette was connected to the inlet so as to admit known quantities of fluid, and the outlet was connected to a sensitive tambour, which actuated a light lever placed in the path of light from the galvanometer so as to throw a shadow on the plate. When the tap between the pipette and the cannula and the outlet tap were both closed, isochoric records were obtained. When the former tap was opened, the records were auxobaric; the movement of the lever in this case was sufficient to indicate the duration of the contraction, but not its amplitude.

(2) *Ventricular strip.* A strip of ventricle was cut from the heart, and a piece of auricle and sinus was left attached in order to provide regular stimuli. A silver hook was inserted into the apex of the ventricle, and connected by a silk thread to the mechanical recorder. This latter was a very light lever mounted on a very fine hair spring. The excursion was recorded optically. The sensitivity of the lever could be brought to an optimum by varying the tension on the hair spring by means of a screw at one end. The lever was calibrated in order to provide known tensions to the strip.

(3) *Auricular preparation.* The ventricle was removed and a calibrated tube inserted into the auricle, to which the sinus was left attached. The filling of the cannula, and hence the pressure, was varied by the addition and removal of Ringer's fluid by means of a fine pipette. A small piece of rubber tubing was placed over the top of the cannula; when this was closed by a clip, isochoric contractions were obtained. There was an air space of about 0·5 c.c. above the fluid in the cannula, and hence the contractions were only approximately isochoric when the clip was closed, but since the auricles only exerted a pressure of about 10 cm. of water, the movements due to the presence of this air volume were not more than about 0·005 c.c.

Influence of variations of initial filling and initial tension on the electrical response.

Starling(7) showed that the force of contraction and the oxygen use of the heart depend upon the initial filling. This subject was further investigated by Clark and White(8), who showed that the oxygen consumption of the frog's heart contracting when empty, was nearly the same as when arrested, whereas when it contracted with an adequate filling it used about four times this quantity. Frank(9) showed that the pressure produced by contraction approaches zero as the initial filling is reduced towards zero. Variations in the initial filling, therefore, provide a ready method of varying the energy liberated by the contraction process, whether this be measured by the mechanical response or by the oxygen consumed.

Our experiments show that, when the filling is varied, the electrical response does not vary in the same manner as the mechanical response. We first tested the effects of variations of the initial filling on the electrical response of the auricle and ventricle with preparations (1) and (3). The height of the electrical response was measured from the top of the base line to the tip of the initial deflection (*R* wave).

A typical record from an auricle (preparation (3)) is shown in Fig. 1. Increasing the filling clearly decreases the height of the electrical response. Fig. 2 shows the same effects obtained with the whole ventricle (preparation (1)). While the height of the mechanical response increases to a maximum with increase in filling, the height of the electrical response decreases. This confirms the results of Straub(10), Seeman(11), Daly(12) and Hafkesbring and Ashman(35), who all found that increased filling decreases the height of the electrical response.

These results are, however, open to the objection that the height of

the electrical response is changed by the volume of fluid with which the heart tissue is in contact. Craib's(13) figures show that the height of

Fig. 1. Effect of emptying on diphasic electrical response of frog's auricle. Auxobaric conditions. Auricle empty at *A*; filling gradually increased between *A* and *B*, maximum at *B*; auricle emptied again between *B* and *C*. Read from right to left. Time marked in seconds in this and in all succeeding records.

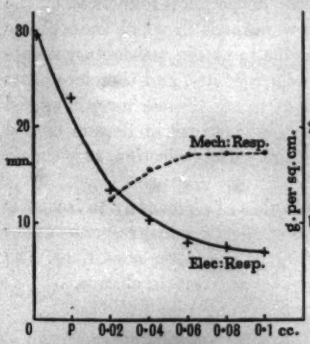

Fig. 2.

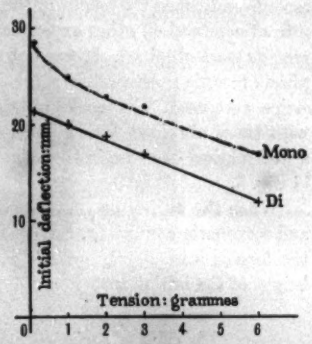

Fig. 3.

Fig. 2. Effect of increased pressure on mechanical and diphasic electrical responses of frog's ventricle. Isochoric conditions. Ordinates: left, height of initial electrical deflection in mm.; right, pressure produced in g. per sq. cm. Abscissa: filling in c.c.

Fig. 3. Effect of increased tension on the monophasic (Mono) and diphasic (Di) electrical responses of frog's ventricle strip preparations. Ordinate: height of initial electrical deflection. Abscissa: tension in g.

the electrical response of a ventricular strip suspended in air is greater than that of a strip on the surface of a considerable quantity of Ringer's

fluid, or than the height of the electrical response of a strip immersed in Ringer's fluid. To avoid this source of error we used a ventricular strip suspended in air (preparation (2)). Increased tension on a strip of ventricle was found to produce the same results as before, namely, a diminution in the height of the initial deflection and in the duration of the electrical response.

The effect of increasing the tension, on the electrical response of heart strips, is shown in Table I. These measurements were on diphasic

TABLE I. Effect of increasing the tension on the electrical response of a strip of frog's ventricle.

Tension in g.	Height of initial deflection of electrical response in mm.	Duration of electrical response		Duration of cardiac cycle	
		(1) In sec.	(2) p.c.	(1) In sec.	(2) p.c.
0	21·5	0·909	100·0	1·25	100
1	20·0	0·857	94·3	1·33	106
2	19·0	0·850	93·5	1·4	112
3	17·0	0·825	90·7	1·4	112
6	12·0	0·824	90·6	1·46	117

records and, since the height of the initial deflection is influenced by the rate of conduction, other experiments were made in which monophasic records were obtained. It was not possible to obtain satisfactory monophasic records from ventricles filled under pressure, and therefore heart strips were used. The results obtained with monophasic records agreed with those obtained with diphasic records; increase in tension caused a well-marked decrease in the height of the initial deflection, as is shown in Fig. 3.

When the auricle or ventricle was subjected alternately to isochoric and auxobaric conditions, the duration of the electrical response under the former was slightly greater than under the latter conditions. The height of the initial deflection did not show any certain change.

The effect of variation in filling on the duration of the mechanical and the electrical responses.

Frank(9) showed that, with increased filling, the initial pressure, the total developed pressure, the effective developed pressure and the duration of the mechanical response increased, but only up to a certain filling, after which the added systolic pressure and its rate of development diminished, while the duration of the mechanical response continued to increase. Segall and Anrep(16) found, with the frog's ventricle, that with increased filling the duration of the rise of the mechanical response

increased until two-thirds of the maximum filling was attained, after which it decreased, whilst the duration of the fall increased.

The measurement of the durations of the mechanical and electrical responses is subject to certain difficulties. As regards the former, the tambour method of recording heart movements usually shows a latent period of more than 0·05 second (Tso(14)). Nearly the whole of this is, however, due to instrumental error, for de Jongh(15) showed that, under optimum mechanical conditions, the commencement of the electrical and mechanical responses of the frog's heart are simultaneous. We made a few experiments with light levers which pressed directly on the heart muscle, and found that the E.–M. interval was not more than 2σ. This arrangement did not give a quantitative measure of the mechanical response, and to obtain this we used preparations (1) and (2), with which there was an appreciable mechanical lag, but to eliminate this error we measured the duration of the mechanical response from the initial deflection of the electrical response.

The determination of the end of the contractile process presents certain difficulties. The mechanical response of the fresh heart shows a sharp rise, followed by a nearly level plateau, which terminates abruptly.

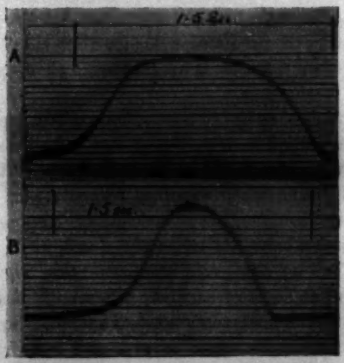

Fig. 4. Effect of prolonged isolation on mechanical response of frog's ventricle. Strip preparation: *A*, fresh preparation; *B*, same preparation 6 hours later. Read from right to left.

This effect is shown in Figs. 4 and 5. It will be seen, from a comparison of these two figures, that the angle of descent is much more marked in

the case of the isochoric record taken with the delicate tambour (Fig. 5) than in the case of the ventricular strip (Fig. 4 *A*). After an hour's

Fig. 5. Mechanical and diphasic electrical responses of frog's ventricle. Isochoric conditions. Read from right to left.

isolation the mechanical response, however recorded, became hump-shaped (Fig. 4 *B*). This effect has been described previously by Junkmann(17) and by Tso(14). Hartree and Hill(18) described an "angle" in the mechanical response of skeletal muscle, and Fulton claimed that it represented the end of the contractile process. Cooper and Eccles(19), however, have recently produced evidence that the "angle" observed in records from skeletal muscle is an artifact, due to mechanical defects in the recording mechanism.

The frog's ventricle, owing to the feeble tensions it produces, and the irregular distribution of its fibres, is a less suitable structure than a skeletal muscle with parallel fibres for the determination of the exact course of events during the mechanical response. On the other hand, the heart has the advantage that its mechanical response lasts from 1 to 2 seconds, which is about ten times the duration of a frog's skeletal muscle twitch. We do not claim any theoretical significance for the occurrence of the "angle" so frequently observed in heart records under widely varying mechanical conditions. The fact, however, that the mechanical response of the heart often shows a nearly level plateau, makes it extremely difficult to measure exactly the moment of maximum tension or contraction. We therefore took the amount at which the descent became rapid as indicating the termination of the contractile process. In a certain number of cases, measurements of the mechanical response were taken both to the summit and to the angle. Such variation in the method of measurement did not affect any of the conclusions we

have drawn regarding the manner in which variations in filling alter the duration of the mechanical response. In practice, however, we found that the angle was the more convenient point of measurement.

The duration of the period of rising and sustained pressure, as measured from the initial electrical deflection to the angle, could be determined exactly (*i.e.* within an error of 1σ), whereas the full duration of the mechanical response could not be measured accurately. The duration of the electrical response was measured from the tip of the initial deflection to the tip of the final deflection. This final deflection could not always be determined accurately. Segall and Anrep(16) concluded that increase of filling of the frog's isolated ventricle increased the periods of rising and falling pressure by about 25 and 30 p.c. respectively. Hafkesbring and Ashman(25) obtained similar results with the turtle's isolated ventricle. These observers measured the period of rising pressure from the commencement of the mechanical record, but Tso(14) found that the apparent latent period is much greater with small than with large fillings, and that measurements of the period of rising and sustained pressure, made from the moment of stimulation, show that this period decreases slightly when the filling is increased. Hafkesbring and Ashman(25) measured the effect of variations of filling on the duration of the electrical response of the turtle's isolated ventricle, and found this to be unaltered when the filling is increased. Our results show that increased fillings cause a slight diminution in the duration of the electrical response of the frog's isolated ventricle.

Tables I, II and III show the results we obtained with three different

TABLE II. Effect of variation in the filling on the electrical and mechanical responses of frog's ventricle (isochoric conditions).

	Electrical response			Mechanical response				
	(1) Height of initial deflection	(2) Duration		Pressure produced	Duration of rising and sustained pressure		Duration of cardiac cycle	
Filling in c.c.	in mm.	(*a*) In sec.	(*b*) As p.c.	(g. per sq. cm.)	(*a*) In sec.	(*b*) As p.c.	(*a*) In sec.	(*b*) As p.c.
Empty	29·5	1·28	86·5	0	—	—	3·55	93·7
0·02	13·5	1·48	100·0	12·5	1·545	100·0	3·80	100·0
0·04	11·0	1·36	92·0	15·5	1·420	92·0	3·70	97·5
0·06	8·0	1·34	90·5	17·0	1·370	88·6	3·60	95·0
0·08	7·8	1·25	84·5	17·0	1·290	83·5	3·40	89·5
0·10	7·0	1·24	83·8	17·0	1·260	81·5	2·98	78·5

preparations. They confirm Tso's conclusion, for increase in filling or tension is in every case associated with a slight diminution in the duration of the period of rising and sustained pressure or tension.

Unfortunately the frequencies of the hearts show variations. These cannot account for the alterations in the duration of the responses, because the frequency diminishes as the tension increases in Table I, whereas it increases as the filling increases in Tables II and III, but the duration of the electrical response diminishes in all three cases.

TABLE III. Effect of variation in the filling on the electrical response of the frog's auricle (Exps. A and B).

Volume in c.c.		Pressure in cm. of water		Electrical response			Duration of cardiac cycle		
Diastolic	Systolic	Diastolic	Systolic	Height of initial deflection in mm.	Duration in sec.	Duration as p.c.	(a) In sec.	(b) As p.c.	Condition
A.									
0	0	0	0	?	0·610	100·0	1·965	100·0	Auxobaric
0·0185	0·009	1·60	1·75	28	0·580	95·0	1·885	96·0	Auxobaric
0·0185	—	1·60	—	26	0·600	98·5	1·865	95·0	Isochoric
0·059	0·022	2·80	3·30	22	0·452	74·1	1·418	72·4	Auxobaric
0·059	—	2·80	—	23	0·510	83·6	1·419	72·3	Isochoric
0·185	0·092	5·20	6·70	19	0·463	76	1·392	71·0	Auxobaric
0·050	0·015	1·90	2·40	23	0·539	88·4	1·512	77·0	Auxobaric
B.									
0·025	0·012(5)	1·60	1·80	32	0·540	100	1·745	100·0	Auxobaric
0·225	0·210	8·30	8·50	16	0·462	85·5	1·660	95·2	Auxobaric
0·225	—	8·30	—	15	0·576	106·8	1·646	94·2	Isochoric

The effect on the electrical response of procedures which depress the frog's heart.

Five procedures which depress the mechanical response of the frog's heart were tested.

(1) *Calcium lack.* With calcium-free Ringer's fluid the mechanical response of the heart can be almost completely abolished without producing any measurable changes in the extent or form of the electrical response. Fig. 6 shows a reduction of 85 p.c. in the height of the mechanical response, while the height of the electrical response is slightly increased. Calcium-free Ringer's fluid produces a slight decrease in the duration of the electrical response.

The above experiment was repeated with the apex of the ventricle, burnt with a red-hot probe in order to obtain monophasic responses. Identical results were obtained, there being no parallelism between the changes in the mechanical and electrical responses. Fig. 7 shows the recovery of the mechanical response after it had been reduced with calcium-free Ringer's fluid. The height of the mechanical response increases nearly tenfold, while the height of the electrical response remains unaltered.

Fig. 6. Effect of calcium lack on mechanical and diphasic electrical responses of strip of frog's ventricle. *A*, normal Ringer's fluid; *B*, calcium-free Ringer's fluid. The deflection produced by 0·2 millivolt is shown at the left of each record. Read from right to left.

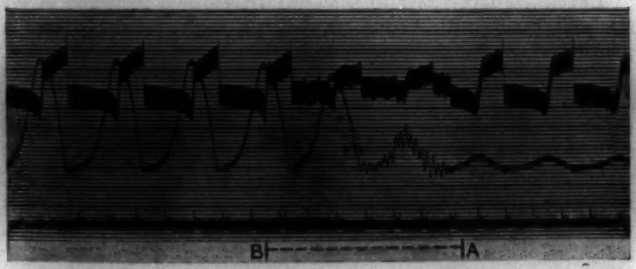

Fig. 7. Effect of changing (at *A*–*B*) from calcium-free to normal Ringer's fluid on mechanical and monophasic electrical responses of strip of frog's ventricle. Read from right to left.

(2) *Acetyl choline* (10^{-6} molar) did not affect the height of the initial electrical deflection even when the mechanical response was reduced to less than 2 p.c. of its normal height. The end deflection, however, was greatly increased in amplitude and reversed. The duration of both the mechanical and electrical responses was considerably diminished. Fig. 8

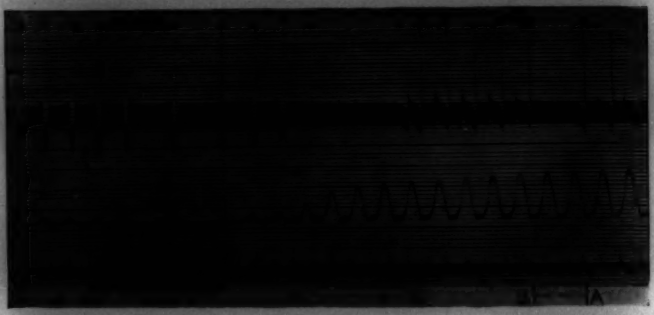

Fig. 8. Effect of acetyl choline (10^{-4} molar added at *A–B* and *C–D*) on mechanical and diphasic electrical responses of strip of frog's ventricle. The disturbance associated with the introduction of the drug interferes with the electrical response, but this returns afterwards to its original amplitude. The tips of the initial deflections are marked with dots. Read from right to left.

shows a reduction in the duration of the electrical response from 0·79 sec. to 0·33 sec. Stronger concentrations (10^{-4} molar) arrested both the mechanical and electrical responses.

Bertha(20) found that with muscarin there is a reduction of the mechanical and electrical responses, but that the two are not reduced in proportion, and that the duration of the electrical response is shortened.

(3) *Excess of potassium.* Einthoven and Hugenholtz(3), and Arbeiter(4), found that the action of excess of potassium is to reduce the amplitude of the electrical response. Daly and Clark(21) also found that excess of potassium considerably alters the shape of the electrical response, and that great impairment occurs in the force of contraction, and in the conduction of the impulse from auricle to ventricle and also within the ventricle. Seliskar(22) showed that excess of potassium reduces the intra-auricular conduction.

The striking difference between the effects of lack of calcium and of excess of potassium (0·06 p.c.) is clearly seen by comparing Figs. 6 and 9. Excess of potassium causes a wholly abnormal electrical response.

Its duration is reduced, but the height of the initial deflection is not usually reduced until a late stage of intoxication. Schutz(23) also found that excess of potassium shortens the duration of the monophasic response of the frog's heart, and noted an increase in the time of rise of the initial deflection, a point that is shown in Fig. 9.

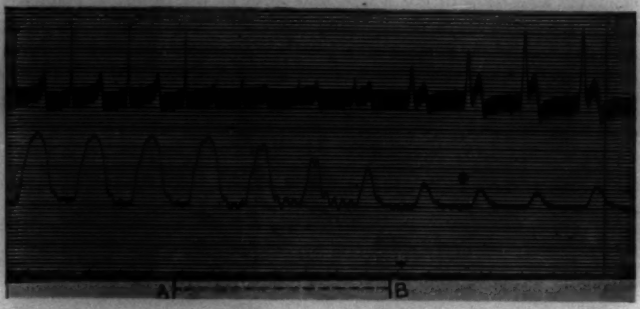

Fig. 9. Effect produced by changing (at *A–B*) from normal Ringer's fluid to Ringer's fluid containing potassium chloride 0·06 p.c. on mechanical and diphasic electrical responses of strip of frog's ventricle. Read from left to right.

(4) *Octyl alcohol* (2×10^{-3} molar) affects the form of the electrical response as soon as it produces marked changes in the mechanical response. The most obvious changes noted were a decrease in the duration of the electrical response as well as an impairment in the rate

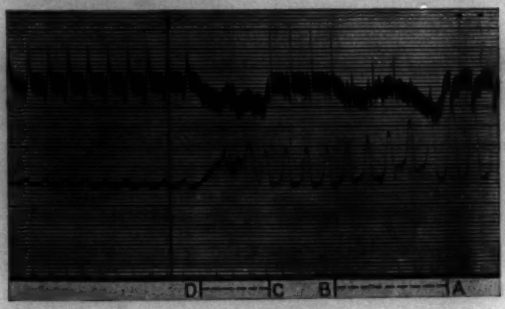

Fig. 10. Effect of octyl alcohol (2×10^{-3} molar) added at *A–B* and *C–D* on mechanical and electrical responses of strip of frog's ventricle. Read from right to left.

of conduction of the wave of excitation. The heights of the electrical and mechanical responses were diminished (see Fig. 10).

(5) *Acid.* (The Ringer's fluid, which was buffered with phosphate, was brought to *p*H 6·0 by the addition of hydrochloric acid.) The acid Ringer's fluid reduced the mechanical response, and also caused considerable alteration in the height and form of the electrical response. The durations of the mechanical and electrical responses were increased, and the rate of conduction was greatly decreased. In some of these experiments, when the heart was nearly dead, the electrical response became polyphasic. Three different stages of the effect of acid Ringer's fluid are shown in Fig. 11.

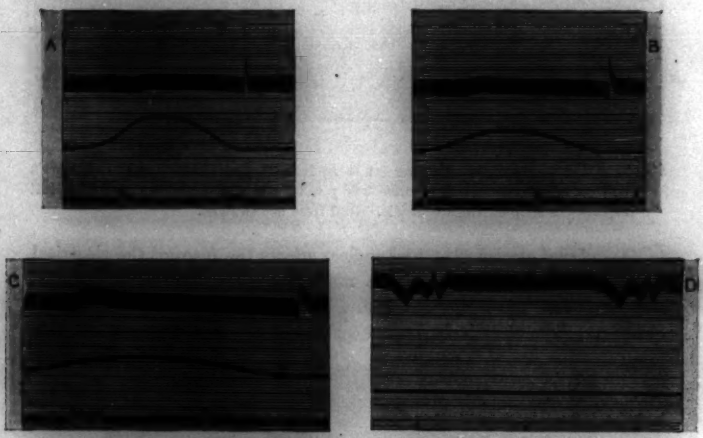

Fig. 11. Effect of acid on mechanical and electrical responses of strip of frog's ventricle. *A*, normal Ringer's fluid; *B*, *C* and *D*, 5, 10 and 20 minutes respectively after commencement of irrigation with acid Ringer's fluid (*p*H 6·0). Read from right to left.

Discussion.

There is no relation between variations in the height of the initial electrical deflection and in the force of contraction, as can be seen from the following summary of the effects of six procedures, all of which reduce the extent of the mechanical response.

	Alteration in the initial deflection of electrical response
Calcium lack	None
Acetyl choline (10^{-4} molar)	None
Excess of potassium	Abnormal
Octyl alcohol	Abnormal and smaller
Acid (*p*H 6·0)	Abnormal and smaller
Emptying the heart	Great increase

No conclusions, however, are drawn as to whether an electrical response can exist without any mechanical response, though the height of the initial deflection definitely remains unaltered, when the mechanical response is reduced to 5 p.c. of the normal. It is possible, therefore, to reduce the height of the mechanical response without affecting that of the electrical response; on the other hand, there is no evidence that the height of the electrical response can be reduced without affecting that of the mechanical response. This is in keeping with the conception of Keith Lucas(24) that a propagated disturbance, accompanied by the electric change, precedes and releases contraction.

Summary.

1. The height of the electrical response and the height of the mechanical response of a frog's heart can vary independently, and therefore the height of the initial deflection is not indicative of the work done by the heart.

2. The height of the initial deflection of the electrical response is diminished when the filling of the auricle or ventricle, or the tension on a strip of ventricle, is increased.

3. The duration of the electrical response, and the duration of the period of rising and sustained pressure are slightly diminished with increased filling or tension.

4. Calcium lack sufficient nearly to abolish the mechanical response does not affect the extent or form of the electrical response. This agrees with the findings of Willigen, but is opposed to those of Arbeiter.

5. Acetyl choline (10^{-6} molar) does not affect the height of the initial deflection, but reverses and greatly increases the amplitude of the final deflection.

6. Octyl alcohol, excess of potassium chloride and acid Ringer's fluid (*p*H 6·0) render the electric response abnormal in form.

We wish to take this opportunity of expressing our thanks to Professor Clark for his instruction and help throughout the course of this work.

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STUDIES OF REFLEX ACTIVITY IN INVOLUNTARY NERVOUS SYSTEM.

II. Action of ergotamine on vaso-motor reflexes.

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Literature and methods.

The action of ergotamine (ergotoxine) on the peripheral vascular mechanism has been carefully studied by Dale(2); he showed that moderate doses of ergotoxine raised the blood-pressure markedly and for a prolonged period owing to a peripherally produced vaso-constriction. With larger doses of the drug the sympathetic vaso-constrictor fibres are paralysed, but the vaso-dilators remain active, giving rise to the well-known splanchnic and adrenaline "reversal" effects. Little attention has hitherto been paid to the effects of this drug on the vaso-motor reflexes. Rothlin(12) noted in the rabbit under urethane anæsthesia that ergotamine abolished the depressor reflex, but he did not investigate the matter further. Heymans and Regniers(8) found that ergotamine in doses which produced adrenaline "reversal" abolished all vaso-motor reflexes arising from the sinus caroticus; they concluded that these reflexes resulted solely from variations of vaso-constrictor tone. The validity of their conclusion is discussed below.

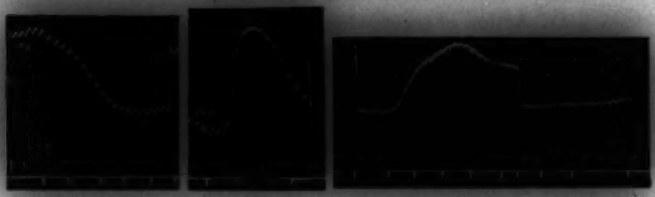
The experiments to be reported here were carried out on the cat under chloralose anæsthesia; both vagi were divided to exclude reflex inhibition of the heart. Pressor reflexes were elicited by stimulation of the central end of the peroneal or popliteal nerves, or by obstruction of one or both common carotid arteries (Hering(6)). Depressor and mixed depressor-pressor reactions were produced by stimulation of the central end of the vagus.

The ergot preparation mainly employed was ergotamine methan-sulphonate. In some experiments ergotamine tartrate or ergotoxine phosphate was used.

Results.

Pressor reflexes.

Small doses of ergotamine (about 0·1 mg. per kg.) prolong the latent period of the pressor reflexes, and decrease the extent and the rate of the rise of blood-pressure. (Fig. 1). In the experiment from which



a *b* *c*

Fig. 1. Cat. In this and all subsequent figures the anæsthetic is chloralose and both vagi are cut. Time in 10 sec. Artificial respiration. (*a*) Control stim. central end of vagus, coil at 7. (*b*) Control stim. central end of peroneal, coil at 7. Between (*b*) and (*c*) 0·2 mg. ergotamine injected. (*c*) Stim. first of peroneal and then of central end of vagus. Subsequently maximal stim. of central vagus was also ineffective.

Fig. 2 was subsequently obtained control strong afferent stimulation (coil at 4) produced a large abrupt rise of blood-pressure (65 mm.);

a *b* *c*

Fig. 2. Artificial respiration (see text). (*a*) Stim. central end of popliteal, coil at 4. After ergotamine (see text). (*b*) Stim. left splanchnic (peripheral end), coil at 10. Adrenals intact. (*c*) Stim. left splanchnic, coil at 10. Adrenal veins tied off.

weaker stimulation (coil at 10) produced a slower and smaller rise of blood-pressure (20 mm.) after a longer latency (3 sec.). 20 min. after the injection of 0·5 mg. ergotamine the latency with strong stimulation was 7 sec. and with weak stimulation 15 sec. Following a further injection of 0·5 mg. ergotamine the latency with strong stimulation was 20 sec. (Fig. 2 *a*), the rate and extent of the reflex rise were much decreased (25 mm.) and the return of the blood-pressure to its previous level was delayed.

If stimulation is discontinued during the latent period no after-effects are obtained. At an early stage of the action of the drug if afferent stimulation is adequately prolonged a reflex rise as great as that obtained prior to the injection may be produced; or it may be found that the pressure may continue to rise (sometimes for as long as 15 sec.) after stimulation is stopped (Fig. 3 *c*). With larger doses of the drug, the

a *b* *c*

Fig. 3. Stim. central end peroneal nerve, coil at 10. (*a*) 30 double shocks per sec. (*b*) 9 d.s.p.s. (3 d.s.p.s. gave a pure fall). Between (*b*) and (*c*) 0·2 mg. ergotamine injected. (*c*) First signal: 30 d.s.p.s. (note after-rise). Second signal: 9 d.s.p.s. (no initial fall but long latency).

pressor reflexes are abolished altogether even to prolonged maximal stimulation of freshly dissected nerves.

The effects described have been observed when the blood-pressure has been at, above, or below the pre-injection level, and with natural or artificial respiration.

Long latent period.

The cause of the lengthening of the latent period of the reflex will first be considered.

(i) Sensory nerves contain an admixture of afferent pressor and depressor fibres. Ergotamine is known to depress the vaso-constrictor nerves and leave intact the vaso-dilators (Dale). It is possible that under

these circumstances the "conflict" between the two sets of afferents might become more evident and the depressor fibres might delay for a time the development of the pressor effect. This suggestion can at once be negatived by the fact that ergotamine abolishes the depressor reflexes before the pressor ones. Figs. 1 *a*, 1 *b* show control stimulation of the central vagus and peroneal nerves. After ergotamine (Fig. 1 *c*) central vagus stimulation, even with maximal currents, produces no effect, while central peroneal stimulation shows the characteristic lengthening of the latent period.

Again, Fig. 3 *b* shows that with infrequent stimulation a mixed effect is produced—an initial fall followed by a rise of pressure. After 0·2 mg. ergotamine the initial fall is abolished, and replaced by a long latent period which passes into a slow rise of pressure (Fig. 3 *c*).

(ii) The long latent period might be due to delay in the peripheral mechanism. This can readily be tested by examining the effect of stimulating the peripheral end of the cut splanchnic nerve. Fig. 2 shows that when the latency of the reflex is about 20 sec., splanchnic stimulation produces an almost immediate effect and the rise of pressure is rapid and considerable. After tying off the adrenal glands (Fig. 2 *c*) splanchnic stimulation still gives an ample pressor effect. Comparison of the latency of the splanchnic rise before and after ergotamine shows that at this stage, and also at a somewhat later stage of the action of the drug, there is no lengthening of this period (Figs. 5 *a* and 6 *a*). These results (which are regularly obtained) show that the peripheral vascular mechanism is still capable of responding rapidly and well both to efferent nervous stimulation and to adrenaline. The long latency of the reflex is apparently due to some action of ergotamine on the central nervous system.

(iii) The state of the vaso-motor "centre" must next be considered. The experiments of Pilcher and Sollmann(11) show that, with doses of ergotamine such as those employed in this study (up to 0·25 mg. per kg.), practically no effects are produced on the tonic discharge of the "centre." The reactivity of the "centre" can be directly tested by subjecting it to acute anæmia. Both vertebral arteries are compressed below the atlas with a McDowall clamp(10), and both common carotid arteries are then clipped. Fig. 4 shows again the long latency of the reflex, and that occlusion of the carotid arteries gives rise to an almost immediate and considerable rise of pressure. The "centre" can thus be made to respond rapidly and vigorously by the stimulus of acute anæmia, and its discharge can find ready access to the spinal sympathetic neurones. As there is

no reason to suppose that conduction in the afferent nerves is in any way altered (*vide infra*), the possible site of the reflex delay is considerably narrowed down. If we assume that the central axons of the posterior

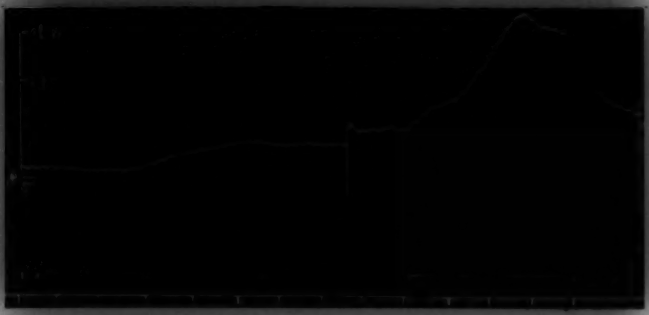


Fig. 4. Same experiment as illustrated in Fig. 2, artificial respiration. First signal: stim. central popliteal, coil at 4. The drum was stopped and the pressure allowed to fall. Vertebral arteries were compressed and pressure rose. Drum restarted and second signal shows period of occlusion of left common carotid artery (arterial cannula in right carotid). At end of signal mark clip was removed from carotid. Finally drum was stopped and vertebral clamp was removed; pressure then fell to the original level.

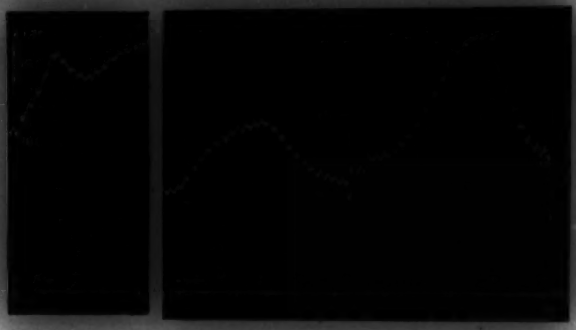
nerve roots pass without relay to the vaso-motor "centre," then the delay would seem to occur just on the afferent side of the "centre."

The cause of the slow and decreased rise of blood-pressure during afferent stimulation will be discussed after certain changes in the peripheral mechanism have been considered.

Loss of pressor reflexes.

The state of the circulation must next be analysed when ergotamine has completely abolished the pressor reflexes. Fig. 6 shows that, at this stage, both splanchnic stimulation and acute cerebral anæmia can still produce a marked rise of blood-pressure. In another experiment, cerebral anæmia at this stage caused a rise of blood-pressure from 90 to 275 mm. Hg (Fig. 6 *a*). These effects of anæmia can still be obtained (though to a lesser extent) after the adrenals have been removed. Ergotamine can thus finally produce a complete "block" in the neighbourhood of the termination of the afferent side of the arc at a time when the effector side from the "centre" onwards is still active.

At this stage certain modifications in the reactions of the blood vessels become evident. Comparison of the splanchnic curves in Fig. 5 *a* and



a *b*

Fig. 5. Artificial respiration. Control curves. (*a*) Stim. left splanchnic, coil at 10. (*b*) First signal: stim. central peroneal, coil at 5. Drum stopped and vertebrals clamped; note rise of B.P. Second signal indicates period of carotid occlusion (complete anæmia). (Cf. Fig. 6.)

Fig. 6 *a* shows that, though the latent period is unaltered by ergotamine, the rate of rise of the blood-pressure is more gradual, though (in this instance) if stimulation is continued long enough a rise as large as that

a *b*

Fig. 6. Artificial respiration. Same experiment as illustrated in Fig. 5 after injection of 0·5 mg. ergotamine. (*a*) First signal: stim. left splanchnic, coil at 10. (Cf. Fig. 5 (*a*).) Second signal: stim. central peroneal, coil at 5. (Cf. Fig. 5 (*b*).) (*b*) Vertebrals clamped with stationary drum. No rise of B.P. Signal indicates period of carotid occlusion. (Cf. Fig. 5 (*b*).)

obtained in the control experiment may finally take place; the return to normal of the blood-pressure is also more gradual. Similar observations have been made by Dale and by van Dyke(5).

It is difficult to determine with certainty the state of the vaso-motor "centre" at this stage. There is some evidence that its sensitivity to the stimulus of acute anæmia may be somewhat diminished. Thus control compression of both vertebral arteries in Fig. 5 *b* caused a rise of blood-pressure of 25 mm. Hg; but after ergotamine (Fig. 6 *b*) this procedure was ineffective (a rise was obtained however in Fig. 6 *a*). Again, with

a *b*

Fig. 6 *a*. (*a*) Control stim. central peroneal, coil at 7. Between *a* and *b* 0·5 mg. ergotamine injected. (*b*) Vertebrals clamped (small rise of B.P.) then carotid clamped (complete anæmia). Rapid rise of B.P. to 275 mm. Repeat stim. central peroneal, coil at 3. No effect.

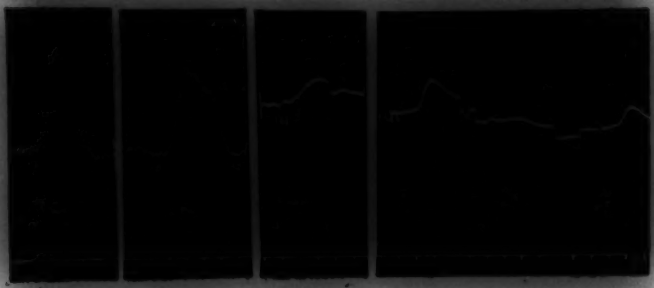
control occlusion of the carotoid arteries in Fig. 5 *b*, the marked rise of blood-pressure occurred after about 10 sec., but after the injection of ergotamine the main rise took place after 20 sec. But as the animals at this stage have often been under the influence of artificial respiration for some time the factor of over-ventilation cannot be wholly excluded (McDowall(10a)).

The cause of the decrease in the rate and extent of rise of the blood-pressure observed in the vaso-motor reflex after ergotamine may now be considered. This would tend to be brought about in part by the peripheral changes which were noted above; but in my experience the decrease in the rate of the reflex rise always develops earlier and more markedly than the similar change in the effector mechanism. It seems therefore that this feature of the reflex response is partly central in origin.

Action on sinus caroticus reflex.

The work of Hering(6) and of Heymans(7) shows that cerebral anæmia produces its effects not only by decreasing the oxygen tension and raising the carbon dioxide tension in the vaso-motor "centre" but also by setting up a pressor reflex from the collapsed sinus caroticus. Anrep and Starling(1) on the other hand argue that the fall in cerebral blood-pressure which results from cerebral anæmia directly stimulates the vaso-motor "centre." The effects of carotid occlusion were therefore studied before and after ergotamine.

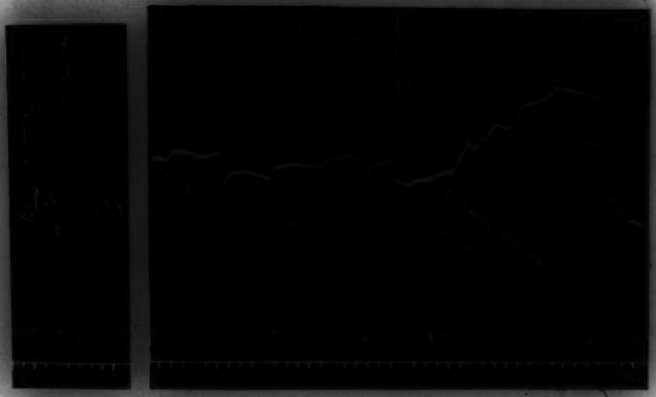
Fig. 7 *a* shows that control occlusion of one carotid artery (into the



a *b* *c* *d*

Fig. 7. Artificial respiration. (*a*) Control occlusion (between points marked by arrows) of right carotid artery (arterial cannula in left; vertebral arteries open). (*b*) Control stim. central peroneal, coil at 5. Between (*b*) and (*c*) 0·3 mg. ergotamine injected. (*c*) Repeat carotid occlusion; the application of the clip is marked on the record by a small sharp rise of pressure which is due to the sudden increase in the peripheral resistance. (Note long latency and slow rise of B.P.) The removal of the clip is for the opposite reason followed by a sharp fall of pressure. (*d*) First signal: stim. central peroneal. Between the vertical lines on the tracing another 0·2 mg. ergotamine was injected. Repeat carotid occlusion (period indicated by arrows): sinus reflex abolished. Second signal: repeat stim. central peroneal: long latency and decreased rise.

other an arterial cannula had been inserted) produces a rise of blood-pressure which is immediate, rapid, and considerable. Repetition after ergotamine causes a small immediate rise which is mechanical in origin; the pressure remains at this level for about 6 sec. and then a slow small further rise of pressure occurs (Fig. 7 *c*). Finally (Fig. 7 *d*), no effect is produced except the small mechanical effects; at this stage central peroneal stimulation shows the characteristic long latent period and the reduced and slower pressor response. It is clear from this experiment that the "natural" sinus caroticus reflex is modified by ergotamine in the same way as the reflexes produced more artificially by afferent nerve trunk stimulation. In my experience it is usual for the sinus reflex to be abolished earlier than the response to strong afferent stimulation. Fig. 8 shows that ergotamine may abolish the sinus reflex



a *b*

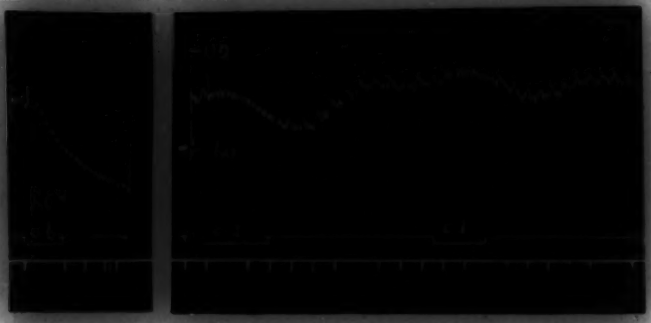
Fig. 8. Artificial respiration. (*a*) Cannula in femoral artery. Control occlusion of both carotids (vertebral arteries open). 0·5 mg. ergotamine injected between (*a*) and (*b*). (*b*) Repeat carotid occlusion twice; on third occasion carotid occlusion was followed by obstruction of vertebrals to produce acute cerebral anæmia.

at a time when acute cerebral anæmia (produced by compressing both vertebrals too) can still produce an immediate rise of blood-pressure. These results obviously bear on the work of Heymans and Regniers[8], who noticed that ergotamine abolished the sinus reflexes when the peripheral vaso-constrictor mechanism was paralysed. Figs. 7 and 8

show clearly that in the cat the sinus reflexes are first lost at a considerably earlier stage.

Depressor reflexes.

Fig. 1 has already illustrated the early abolition of the depressor reflexes by ergotamine, and confirms for the cat Rothlin's original observation in the rabbit. When smaller doses of the drug are used, it is found that the depressor reflexes are modified in the same way as the pressor—namely, the latency of the reflex is increased and the rate and extent of the fall of blood-pressure are slower (Fig. 9). The recovery of the blood-pressure to normal may be more rapid than in the control curves. In Fig. 9 these changes are noticed after ergotamine, although



a *b*

Fig. 9. Artificial and natural respiration. (*a*) Stim. central vagus, coil at 6. Inject 0·02 mg. ergotamine. (*b*) Stim. repeated, coil at 1. Note longer period of latency and smaller fall of pressure with second stim. compared with first, and of both compared with control in (*a*).

stronger stimulation was employed than in the control curve. In trying to account for the above results, attention must be paid to the following points.

(i) The depressor reflex involves central inhibition of vaso-constrictor tone and stimulation of vaso-dilator nerves (Bayliss). The sympathetic vaso-dilators as shown by Dale(2) are active after the sympathetic vaso-constrictors are paralysed, and they may be presumed to be intact after the small doses of ergotamine used in this study. The posterior nerve root dilators are believed to act by liberating chemical agents peripherally, possibly acetyl-choline (Dale(3)), or histamine(9). Rothlin

found that after ergotamine there is increased reactivity to acetylcholine, and Fig. 10 shows that, after abolition of the depressor reflex, the fall of blood-pressure produced by histamine is not less great than that observed in the control experiment.

(ii) Inhibition of the vaso-motor "centre" might fail to cause relaxation of the blood vessels, if the latter were "stiffened" by the action of a peripheral vascular stimulant. But ergotamine abolishes the depressor reflex when very small doses are employed which produce only pure transient falls of blood-pressure, and when the blood-pressure is at or below the pre-injection level (Fig. 10). Furthermore, in my experience,

a *b* *c*

Fig. 10. Artificial respiration. (*a*) Control stim. central vagus. (*b*) Control injection 0·01 mg. histamine base (first arrow) and 0·01 mg. ergotamine (second arrow) which produced a fall of pressure. (*c*) Repeat stim. to central vagus; smaller rise and fall during stimulation and smaller and slower after-fall compared with (*a*). At first arrow inject 0·01 mg. ergotamine which gave a greater fall of pressure than in (*b*), and at second arrow repeat injection 0·01 mg. histamine which gives a fall not less marked than control. Second signal is repetition of central vagus stim. Slight stimulatory rise but no fall or after-fall.

the depressor reflex can still be obtained during the hypertension produced by vasopressin.

(iii) As the depressor reflex may be abolished by doses of ergotamine of 0·01 mg. per kg. we can be fairly certain, in the light of Pilcher and Sollmann's work (11), that the tone of the vaso-motor "centre" has not been affected by the drug.

The failure of the afferent vagus stimulation to cause inhibition of the vaso-motor "centre" and to stimulate the vaso-dilators would seem to be due, therefore (as in the case of the pressor reflexes), to a disturbance on the afferent side of the reflex arc.

Frequently afferent vagus stimulation produces a mixed pressor-depressor effect—as a rule an initial rise followed by a fall. Appropriate doses of ergotamine may then cause a decrease in the depressor part of the reflex and finally cut it out altogether, leaving a pure pressor response

(Fig. 11). This pressor reaction may actually be greater than that seen in the control curve, presumably owing to elimination of the simultaneously operating and competing depressor influence; but if the dose

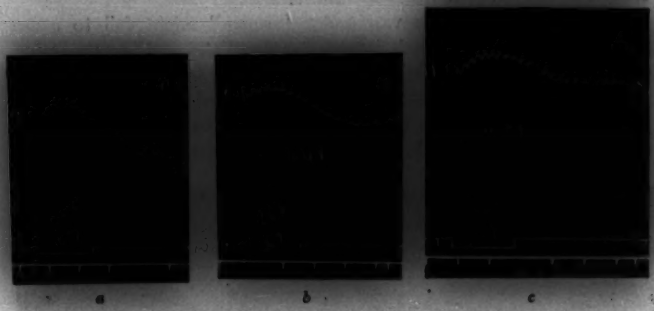


Fig. 11. Artificial and natural respiration. (*a*) Stim. central end vagus, coil at 7. 11.45 a.m. inject 0·02 mg. ergotamine. (*b*) 11.48 a.m. repeat central vagus stim. (*c*) 11.57 a.m. repeat stim.

of ergotamine employed is such as to modify the pressor reactions too, then the rise may be slower in onset, development and in extent (as with the somatic pressor reflexes). Similarly when afferent vagus stimulation gives a pure rise of pressure followed by an after-fall on cessation of stimulation, ergotamine may abolish the after-fall and leave the stimulatory rise.

The depressor reflexes may be weakened or abolished by doses of the order of 0·01 to 0·02 mg. per kg. With these very minute doses the effect of the drug reaches its maximum after a latent period of some minutes, *e.g.* in Fig. 11 the depressor reflex was weakened after 3 min. and was abolished 9 min. later; similarly gradual recovery usually occurs.

Nature of ergot preparation employed.

As stated, in most of the experiments, ergotamine salts (Sandoz) have been used; but the principal results described have also been obtained when ergotoxine (Burroughs, Wellcome and Co.) was employed. This lends additional support to the view of Dale and Spiro(4) that there is no essential difference in the physiological action of the two preparations.

Importance of chloralose.

I have not been successful in repeating these experiments in the decerebrate preparation. It is possible that chloralose favours the development of the phenomena described by depressing central processes (Wright(16)), and by stimulating the sympathetic(15). It would thus counteract the peripheral action of ergotamine and provide favourable conditions for the display of its central action. In support of this suggestion is the fact that, in my experience, very much smaller doses of ergotamine (*e.g.* 0·1 mg. per kg.) completely paralyse the vaso-constrictors in the decerebrate preparation.

Action of strychnine.

As strychnine is widely supposed to act in an excitant manner on the afferent side of the reflex arc (in the same region that it is suggested that ergotamine exerts its depressant action), attempts were made to restore with it vaso-motor reflexes which had been weakened by ergotamine, but without success even when just-convulsive doses of strychnine were used.

Action of ergotamine on other reflexes.

(1) *Respiratory reflexes.* The apnœa resulting from central vagus stimulation persists even after doses of ergotamine, which seriously depress the respiratory centre. Thus, in Fig. 12, after ergotamine,

Fig. 12. Respiration—inspiration = upstroke. Vagi cut. (*a*) Inject 0·35 mg. ergotamine (0·4 mg. previously given in divided doses). Breathing becomes markedly apneustic with superimposed gasps, and finally purely gasping. Artificial respiration was put on for 5 min. and natural breathing returned. (*b*) Stim. central vagus, coil at 5. Apnœa and post-stimulatory apnœa (identical with that obtained prior to ergotamine).

apneustic and then gasping breathing took place and finally breathing ceased altogether. Natural breathing was restored by a short bout of artificial respiration; it was then found that central vagus stimulation arrested breathing as effectively as in the control experiment.

(2) *Skeletal reflexes.* The flexor and crossed extensor reflexes are readily obtained in the decerebrate animal after the vascular reflexes have been abolished. The action on the muscular reflexes has not been analysed in any further detail, but the observation shows that, at this stage, conduction in (at any rate some of) the afferent nerves has not been interfered with.

(3) *Cardiac reflexes.* The effect of ergotamine on these reflexes was studied by stimulating the central end of one (cut) vagus to produce reflex slowing; the other vagus was left intact, and was stimulated in continuity to measure peripheral excitability. The problem is complicated by the fact that ergotamine heightens peripheral vagus excitability (Dale(2), Rothlin(12)) and markedly slows the heart. In my experience the cardiac reflexes persist after doses of ergotamine more than sufficient to abolish vaso-motor reflexes. In some experiments (with larger doses) reflex slowing could not be obtained when the peripheral vagus was still excitable, but this effect may be due to paralysis of the vagus centre (Sollmann).

Discussion and conclusions.

1. It has been shown that small doses of ergotamine prolong the latency of the pressor reflexes when there is no alteration in peripheral latency, when splanchnic stimulation can still produce an almost immediate rise of blood-pressure and when the vaso-motor "centre" can react rapidly and strikingly to the stimulus of cerebral anæmia; it was argued that the delay must be located in the immediate vicinity of the afferent side of the "centre." To produce effects at this site doses of the order of 0·1 to 0·15 mg. per kg. body weight must usually be employed, which exert little peripheral depressant action.

If direct transmission of the nervous impulse through the "centre" is presumed, and the delay is attributed to slowed conduction in the "synapses" on the afferent side of the "centre," and if the length of a "synapse" is as great as 1 μ, then, to account for a central delay of the order of 20 sec. on this basis would necessitate the assumption that the rate of conduction in the synapse had fallen to 1 mm. in 20,000 sec. or 100 metres in 2×10^9 sec. (over 100 years)! Or if one assumes that

the "synapse" is an area of decrement, that the initial impulses fail to pass through, and that succeeding impulses always arrive in the "supra-normal" phase and proceed a little further before being extinguished, it still means (when stimulating at the rate of 100 per sec.) that some 2000 impulses must each "facilitate" the passage of their successor before an impulse can finally bridge the synapse and arrive at the "centre." Further it has been shown that the same type of central delay is obtained whether infrequent (Fig. 3) or rapidly interrupted afferent stimulation is employed, and even the difficult presumptions just made fail to explain the fact that the rate and extent of the reflex rise of blood-pressure are decreased more than is accountable by the peripheral changes.

In the above argument, one could substitute for "synapse" an altered state of the surface membrane of the central cells, without affecting the subsequent reasoning, so long as direct transmission from the afferent to the efferent side is accepted.

The results described can more readily be accounted for, if one assumes with Sherrington(13, 14) that the afferent nerves liberate at their central terminals an exciting agent (*E*), or set up a "central excitatory state" which is of a cumulative character, and which must reach a certain threshold value before it can set up a nervous discharge from the centre.

The long latency of the reflex after ergotamine could then be perhaps accounted for in one of two ways: (i) that ergotamine depresses the development of the exciting agency, and that consequently a considerably longer interval than normal must elapse before a liminal concentration is established; (ii) that the drug alters the surface membrane of the central cells, so that again a longer period must elapse before a concentration (higher than normal) adequate to penetrate the resistant layer is reached. Against (i) is the fact recorded above that, in my experience, ergotamine exerts its peculiar action on the afferent side of the vaso-motor "centre" only, and not on other medullary or spinal "centres"; hypothesis (i) therefore involves the further suggestion that the exciting agency in the case of the vaso-motor "centre" is perhaps different from that for other "centres." The second suggestion may be tentatively preferred, and one would suppose that the drug acted selectively on the membrane of the cells of the vaso-motor "centre." Anyway, the conclusion seems inescapable that the long latency involves some central *addition latente*.

Having assumed a surface barrier between the central excitatory state and the cytoplasm of the vaso-motor "centre," and proceeding along the lines of Sherrington's theory, the slower reflex rise after

ergotamine would be due to more gradual "recruitment" of successive units in the "centre," and the smaller rise to extinction of the fringe of the central effect. The final abolition of the reflexes would be due to the barrier becoming so great that it could not be penetrated even by maximal concentrations of exciting agency.

Acute cerebral anæmia can still produce its usual stimulating action, because (unlike the exciting agency produced by afferent nerves) it acts within the cell membrane by setting up intracellular metabolic disturbances. It was pointed out that larger doses of ergotamine lessened the reactivity of the centre to acute anæmia; this might be due in part to the drug later modifying the cytoplasm of the cell.

2. In a previous paper I have shown that increasing the strength of afferent vagus stimulation shortens the latency and increases the rate and extent of the fall of blood-pressure. It has been shown that ergotamine affects the depressor reflex in exactly the reverse direction, and that it probably produces its results by a central action. This action could readily be accounted for by assuming either that ergotamine depresses the formation of the central inhibitory agent and finally suppresses it altogether, or better (for the reasons just considered in (1)) that it alters the cell membrane in such a manner as to prevent access of the inhibitory agent. But as the vagus contains an admixture of afferent depressor and pressor fibres, and as ergotamine weakens depressor reflexes earlier than pressor reflexes, an additional explanation of the longer latency of the depressor reflex cannot be excluded, namely that owing to weakening of the depressor component the pressor elements operate more effectively and so delay for a time the reflex fall of blood-pressure (this, however, cannot account for the complete abolition of the reflex).

It is noteworthy that ergotamine deals similarly with the fall and after-fall of blood-pressure which may result from afferent vagus stimulation; this suggests that both are produced by similar mechanisms.

3. Heymans' and Regniers' conclusion that the sinus caroticus reflexes depend solely on variations of constrictor tonus is not justifiable from their experiments on the cat, because it has been shown above that these reflexes are abolished by a central action of the drug when the vaso-constrictors are still intact[1]. The fact, too, that after ergotamine,

[1] *Note added in proof.* After my results were demonstrated to the Physiological Society, Heymans, Regniers and Bouckaert (in unpublished experiments) re-investigated the action of ergotamine on the sinus reflexes of the dog. In this animal they find that ergotamine first suppresses the sinus reflexes by its peripheral action;

lowering cerebral blood-pressure (*e.g.* by carotid occlusion) fails to stimulate the vaso-motor "centre"—when the drug has mainly involved vascular reflexes and the "centre" is sensitive to acute complete anæmia—argues somewhat against Anrep and Starling's view that the vaso-motor "centre" is directly affected by such changes of pressure.

Summary.

1. In the vagotomized cat under chloralose anæsthesia, ergotamine in small doses prolongs the latent period of the depressor reflex, decreases the rate and extent of the fall of blood-pressure and finally abolishes the reflex completely. When central vagus stimulation gives a mixed effect (initial rise followed by a fall or after-fall) ergotamine may convert the response into a pure rise.

2. With larger doses the pressor reflexes are affected. The latent period is prolonged, the rate and extent of the rise of pressure are decreased and finally the reflex is completely abolished. At this stage there is no increased latency of the peripheral vascular mechanism and the vaso-motor "centre" can respond strikingly to acute complete anæmia.

3. Evidence is offered that the effects described are mainly due to an action on the afferent side of the vaso-motor "centre."

4. The pressor sinus caroticus reflex is affected in the same way as artificially produced pressor reactions, and for the same reasons.

5. It is suggested that the results obtained are opposed to the view of direct transmission of the excitation process through the vaso-motor "centre." If Sherrington's conception of central excitatory and inhibitory states is adopted, the results may be accounted for by supposing that ergotamine prevents access of these states to the cells of the "centre," possibly by modifying the condition of the surface membranes of the cells.

I am indebted to Messrs Sandoz for supplies of ergotamine. The expenses of this research were partly defrayed by a Government Grant through the Royal Society.

larger doses (perfused through the isolated head) also abolish the reflexes by an action on the vaso-motor centre. The relative sensitivity of the centre and periphery to ergotamine in the dog appears to be different from that found in the cat.

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THE INFLUENCE OF VITAMIN A DEFICIENCY ON THE ŒSTROUS CYCLE OF THE RAT. II.

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It was shown by one of us(1) early last year that the continued occurrence of cornified cells in the vaginal smear of a rat did not always accompany the exhaustion of the animal's vitamin A reserves, as indicated by its cessation of growth. It was, therefore, concluded that it was not possible to rely on this symptom for diagnostic purposes, as claimed by Evans and Bishop(4).

Later work in these laboratories(2, 3) has led us to wonder whether the cessation of growth in our vitamin A experiments had always been due to a lack of vitamin A or to a lack of some other factor. We have found that many of our animals used for vitamin A tests and given even large doses of cod-liver oil ceased to grow after some time, and we concluded that their diet was lacking in some factor hitherto unrecognized. The required factor was found first in light-white casein (a B.D.H. preparation), and, later, in other food substances such as milk, beef muscle and liver, wheat embryo, green grass, etc. It was not found in butter, marmite (a rich source of vitamins B_1 and B_2), a "crude" casein of B.D.H. manufacture, beef fat ("suet"), etc. The extraction of light-white casein, once with alcohol and four times with ether in the cold, did not remove it. Comparisons were then made of extracted light-white casein and vitamin-free casein ("Glaxo") as the source of protein in a vitamin A test of cod-liver oil. It was obvious that the "vitamin-free" casein ("Glaxo") was lacking in some factor which the extracted light-white casein contained, or, alternatively, it was a protein of "poorer" quality than the other. We inclined to the former belief, for many of our animals given a good pre-experimental diet grow as well on the vitamin-free casein ("Glaxo") as on the extracted light-white casein, and we consider that these animals have made sufficient reserves of the unknown

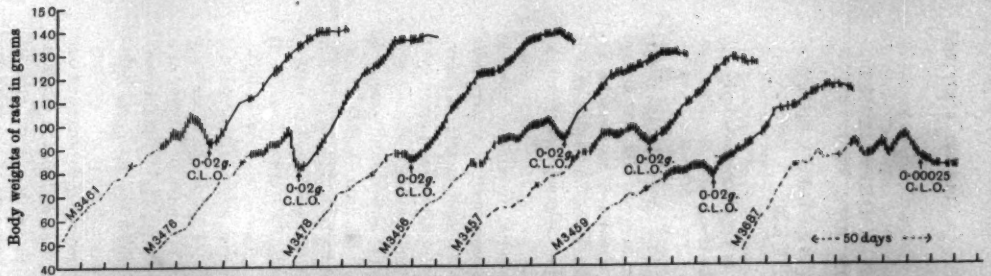

Fig. 1. *Group* 1. To show that the continuous occurrence of cornified cells in the vaginal smears of rats sometimes precedes the cessation of growth indicative of the exhaustion of their reserves of vitamin A.

Broken line, vitamin A-free diet ad lib.; continuous line, daily dose of cod-liver oil given also; ×, opening of the vagina; ✻, death of the animal. Each upright line passing through the growth curve signifies the occurrence of nucleated and cornified cells only (no leucocytes) in the vaginal smear for one day. A shorter upright line, pendant from the growth curve, signifies a mixture of cornified cells and leucocytes.

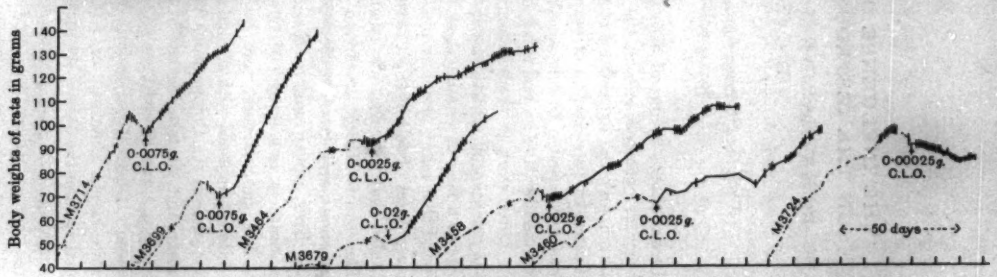

Fig. 3. *Group* 3. To show that the continuous occurrence of cornified cells in the vaginal smears of rats does not always accompany the cessation of growth due to lack of vitamin A.

factor. Whichever should prove to be the cause of the different results, it seemed at once to be necessary to repeat our work on the influence of vitamin A deficiency on the œstrous cycle of the rat, substituting extracted light-white casein for the vitamin-free casein ("Glaxo") in the basal diet. We therefore examined by the vaginal smear method the œstrous cycles of rats which were being used for a test of the vitamin A content of a sample of cod-liver oil. The general management of the experiment was exactly as described in the former paper by Coward(1), and the only difference in the diet was the use of extracted light-white casein instead of the vitamin-free casein ("Glaxo"). The diet was still irradiated after the inclusion of the casein (for the generation of vitamin D), as we had shown that the irradiation of light-white casein did not destroy the particular growth-promoting property which we were investigating.

Results.

The results are easily classified in three groups:

Group 1. The rats ceased to grow some time after the vagina opened; in these we found no normal cycles, but, after some days, cornified cells occurred continuously for as much as 21 days before we considered the rats steady in weight and ready to be given the dose of cod-liver oil. After the giving of cod-liver oil, regular cycles were sooner or later resumed. This result is seen in Fig. 1. It confirms the finding of Evans and Bishop(4), and of Macy, Outhouse, Long and Graham(5).

Group 2. The rats became steady in weight; they were given cod-liver oil and they grew for 8 to 17 days before the vaginas opened. In these rats regular cycles were not established for some time after the giving of cod-liver oil, leucocytes only appearing in the vagina for about 14 days. This result is seen in Fig. 2. It is exactly what we found sometimes in our previous work when we used "vitamin-free" casein ("Glaxo"), and confirms our conclusion that it is impossible to rely solely on the continuous occurrence of cornified cells in the vagina as a criterion of vitamin A deficiency.

Group 3. The vaginas of the rats opened some time before growth ceased, but few cycles occurred and there was no long period of continuous occurrence of cornified cells. Thus vitamin A deficiency completely failed to manifest itself by this symptom. The results are shown in Fig. 3.

As normal cycles were established in all animals in time and rates of growth approximately proportional to the dose of cod-liver oil were maintained, it is assumed that the irregularity in cycles noted were due

only to vitamin A deficiency. The improvement in our vitamin A technique has, therefore, confirmed our previous conclusion that the continuous occurrence of cornified cells in the vagina is not a regular accompaniment of the exhaustion of the vitamin A reserves of the rat.

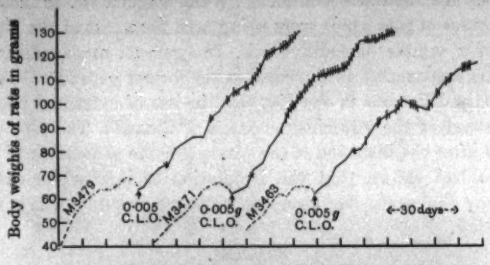

Fig. 2. *Group* 2. To show the cessation of growth from exhaustion of vitamin A reserves before the opening of the vagina.

SUMMARY.

The substitution of extracted light-white casein (B.D.H.) for the "vitamin-free" casein ("Glaxo") previously used in these laboratories has ensured, in vitamin A studies, a cessation of growth due only to a lack of vitamin A.

A repetition of our work on the influence of vitamin A deficiency on the œstrous cycles of the rat, using the improved technique, does not in any way contradict our earlier conclusion. It enlarges our experience of our inability to rely upon the continuous occurrence of cornified cells in the vagina as a criterion of vitamin A deficiency.

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THE EFFECTS PRODUCED BY UNILATERAL LUMBAR SYMPATHECTOMY.

I. Cutaneous temperatures and the limb musculature.

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Within recent years the operations of lumbar ramisectomy or ganglionectomy have been carried out frequently for spastic conditions of the limb and vascular musculatures in man. From the physiological standpoint the possibility of improvement in the former appears to be much less than in the latter. The problem of the relationship of the somatic spinal and of the sympathetic innervation to the tone of the skeletal musculature has been fully discussed by Fulton(1), Forbes(2), Kuntz and Kerper(3), Cobb(4) and many others, and the general conclusion arrived at appears to be that the part played by the sympathetic innervation in the maintenance of skeletal muscle tonus is insignificant, compared to the influence exercised through the reflex somatic spinal pathway. As the controlling influence over the vascular musculature is, on the other hand, essentially a sympathetic or humoral one, such slight disturbances in skeletal muscle tonus as have been described after sympathectomy, might well be produced by metabolic changes resulting from the disturbed vascular control, or from a direct, so-called trophic effect, exercised by the post-ganglionic, non-myelinated fibres on the skeletal muscle fibres. The study of the duration and degree of the cutaneous temperature changes in peripheral parts after interruption of the pre- and post-ganglionic pathways will certainly prove of value when the metabolic changes in the muscles are being subsequently investigated. In the first part of our communication attention will, therefore, be directed to those changes in vascular adjustment, as well as to the general question of the development of the limb musculature in sympathectomized growing animals. As regards vascular adjustments after interruption of the sympathetic pathway, the question arises as to the degree of compensation which can be effected through mechanisms other than the

one which has been interfered with. Thus, in the operation of lumbar sympathectomy, leaving the sacral ganglia intact, there remains a possible post-ganglionic control, and when this is also withdrawn, there remain the local mechanisms concerned with the inherent smooth muscle tonus, associated with an adrenaline action on the denervated vessel wall. The recovery of cutaneous vascular control is evidently more complete and more rapidly effected after sympathectomy in laboratory animals than in man. Thus Brown and Adson(5) observed a distinct cutaneous temperature rise in peripheral parts, after lumbar sympathectomy, in a case of Raynaud's disease, for 3 years after the operation. The average increase in temperature in the feet, after the operation for this vaso-spastic condition, amounted to as much as 12°, and in a case of spastic paralysis a post-operative increase of 5·2°. In the vaso-spastic condition, prior to the operation, there was a range of temperatures from 15° to 31·2°, while after the operation the range under changing conditions was a very limited one, from 32·7° to 36·6°. In the cat and dog the disturbance in vascular adjustment appears, from general references in the literature, to be a more transient one.

In order to investigate possible metabolic changes in sympathectomized skeletal muscle, it was necessary to examine the muscles at different periods after the operation, and also to study the effects produced by exercise, hence a larger number of animals required to be examined than for the study of the vascular changes. The changes produced in cutaneous temperature adjustments were of much the same type in all the animals, so that out of eleven animals operated upon (six puppies and five fully grown dogs) it is only necessary to refer to five.

Operation. The left lumbar cord was exposed by the usual retro-peritoneal approach. After a morphine-atropine injection and while under chloroform-ether anæsthesia, an oblique lumbo-inguinal incision beginning at the costo-vertebral angle was made, and after severance of the different muscle layers, the transversalis fascia was incised without injury to the peritoneum. The peritoneum with the underlying viscera was gently depressed by warm sterilized gauze. After exposure of the medial psoas border, the muscle was gently retracted and the cord readily exposed from diaphragm to pelvic brim. The cord in the neighbourhood of the uppermost large ganglion was caught by forceps, severed just above this point and then gradually freed by careful section of the rami down to the last lumbar ganglion at the pelvic brim. The cord was cut just beyond this point and removed for histological examination.

All the animals recovered rapidly and, apart from a mild diarrhœa of about a week's duration or less, appeared to be absolutely normal, so far as the limb musculature was concerned.

Cutaneous temperatures were determined by the usual thermo-electric method using copper constantan couples, one in a Dewar flask (containing petrol) kept in a microscope box surrounded by cotton-wool packing, the other fixed to a small vulcanite holder. The latter was applied to the interdigital skin, or to the skin at the base of the main cushion pad of the foot.

A description will first be given of the effects produced by this operative procedure on a very young puppy which was kept for 5 months after the sympathectomy.

1. Black spaniel puppy, 4 weeks old, and 1500 g. in weight. Left cord removed on January 18th, 1929, and killed rather more than 5 months later. Before the operation the digital temperatures (with toes held apart) were 30·0° (Right) and 29·7° (Left). As the lower part of the cord was being freed, the temperatures were 30·3° (R.) and 28·4° (L.). One hour after the operation, when the animal had recovered from the anæsthetic, the temperatures were 30·1° (R.), 32·4° (L.).

Fig. 1 *A* gives the temperature graphs for the normal (N.) and

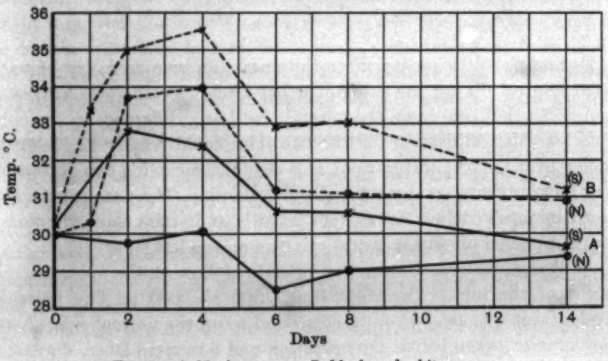

Fig. 1. *A*, black puppy. *B*, black and white puppy.

sympathectomized (S.) sides for the first fortnight. During this period the temperature gradually fell on the sympathectomized side. At the outset the difference between the two sides was about 3°, towards the end 1° or less. These temperatures were taken after the puppy had been

resting for some time beforehand. The absence of a vaso-constrictor response to cold on the left side was easily shown by taking the temperatures after the puppy had been playing about on the cold floor of a basement room. For example, on the fifteenth day after the operation, the temperatures before exercise were approximately the same on the two sides (29·7°); after a few minutes' exercise the temperature on the right side fell to 27·9°, while that on the left side remained unaltered. During the following month exercise of this kind always showed up the difference in adjustment on the two sides, but during the next 3 months this became less evident. The delay in vascular adjustment could readily be shown, however, by warming the feet and legs (dry heat 38°) for 5 minutes, and then exposing the warmed parts to the room temperature (12°–13°) for another 5 minutes. The effects produced on the pad temperatures at different periods are shown in Table I.

TABLE I.

Period after operation (weeks)	Pad temperature before warming		Pad temperature 5 min. after a 5 min. warming period	
	R.	L.	R.	L.
6	30·2°	30·5°	30·4°	33·4°
7	33·0°	31·5°	31·7°	34·1°
11	27·3°	27·4°	27·3°	29·1°
15	29·5°	28·7°	29·4°	30·8°

During the fifth month after the operation, the recovery of vaso-motor control during the cooling period after warming was almost as good on the left side as on the normal one, but a difference in the cutaneous vascular adjustment on the two sides could readily be shown by placing over the feet cotton-wool, and over this a cooling cap of lead-foil containing carbonic acid snow and ethyl chloride. The vaso-constriction after 1 minute's cooling was evident for at least 10 minutes on the normal side, but passed off within 2 or 3 minutes on the left side.

Limb musculature. During the 5 months following the operation, the weight of the puppy increased from 1500 to 9000 g. The muscular development was as good on the operated as on the normal side. X-ray photographs, taken before the operation and 5 months later, showed no differences between the bones and joints of the two sides. Various measurements were taken of the hind limbs before the operation, 2 months and 5 months later, and these may be given to illustrate the development on the two sides. Similar measurements were taken in the case of the other puppies, but, as all showed practically equal growth on

the two sides, it is only necessary to give the figures for this particular animal (Table II).

TABLE II.

	23. i. 29		26. iii. 29		13. vi. 29	
Circular (in cm.):	L.	R.	L.	R.	L.	R.
Thigh	10	10	17·2	17·4	19·7	19·4
Knee-joint	10	10	13·4	14·0	15·8	15·6
Leg	5·5	5·5	7·5	8·5	8·2	8·9
Ankle-joint	7	7	10·1	10·0	10·5	11·2
Foot	7	7	10·0	10·1	11·6	11·1
Longitudinal (in cm.):						
Femur	9	9	10·7	10·6	13·2	13·4
Tibia	6·5	6·5	11·0	11·5	13·9	14·0
Foot	9	9	13·0	13·0	14·7	14·0
Volume (c.c.):	—	—	80	86	188	192

No definite qualitative or quantitative differences between the muscle of the two sides could be observed from galvanic stimulation. A current of 1·5–2·8 milliamps. (cathodal closure) was effective when applied over cutaneous motor points (flexor digitorum). The chronaxie values of the gastrocnemii were determined at intervals. Small skin incisions were made over the muscle, so that the silver-silver chloride electrodes might be brought into contact with the muscle. The values (stated in microfarads) varied within the following limits during the last 3 months—left gastrocnemius 0·034–0·149, right gastrocnemius 0·031–0·061. Such slight differences as were frequently observed most probably were due to certain disturbing factors, for example, local variations from cooling. No differences could be observed in the respective knee jerks, nor could any distinct differences in the muscular tone be detected when the limbs were supported with the puppy lying on its back, and the support gently withdrawn.

2. Black and white puppy, 3 months old, and 1720 g. in weight. Left lumbar cord was exposed on June 28th, 1929, and the lumbar ganglia crushed with forceps instead of the normal procedure of removal of the cord. The animal was killed on November 11th, 1929, the weight then being 5030 g. The development of the muscles, bones and joints, and the growth of hair over shaved patches were, in all respects, the same on the two sides. No constant differences could be made out between the muscles of the two sides on testing with the galvanic current, nor were the chronaxie values different.

Cutaneous temperatures. Fig. 1 *B* gives the course of the temperature changes during the fortnight after the operation. As was the case with all the animals operated on during the summer, the temperature differences between the two sides were less marked than in winter.

3. Brown puppy, 3 months old, and 5900 g. in weight. Left lumbar cord removed on August 23rd, 1929. The digital temperatures for the first fortnight after the operation on the normal (N.) and sympathectomized (S.) sides are given in Fig. 2. The room temperatures during this

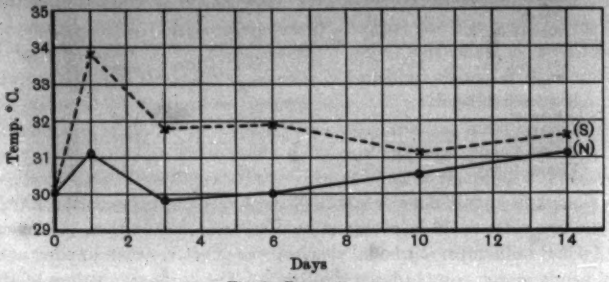

Fig. 2. Brown puppy.

period ranged from 18°–20°. Subsequent to this period, if the temperatures were taken when the animal had been resting for some time, there was but little difference between the two sides (0·5°–1·0°), but the absence of the reflex vascular adjustments on the operated side could readily be shown during a post-exercise period.

From the forty-eighth day after the operation the animal was exercised in a large treadwheel for 10-minute periods four times daily. The stepping rate was 120–140 per minute and, as the wheel was a heavy one, at the end of each exercise period there were evident signs of fatigue, but no difference could be detected between the condition of the muscles on the two sides. The digital temperatures which follow were obtained (*a*) before, and (*b*) and (*c*) 1 minute and 8 minutes respectively after the exercise.

Days after operation	Room temperature		Right	Left
51	12·2°	(*a*)	20·2°	22·7°
		(*b*)	32·3°	31·9°
		(*c*)	22·0°	29·1°
58	14°	(*a*)	26·7°	28·3°
		(*b*)	34·2°	34·3°
		(*c*)	26·8°	29·6°
75	13°	(*a*)	23·3°	24·7°
		(*b*)	34·0°	34·2°
		(*c*)	24·6°	29·1°

For a short period after the exercise the temperatures on the two sides were approximately the same, but in 5 to 10 minutes, especially

if the room temperature were low, the return of vaso-constriction on the normal side was much more evident than on the operated side.

Limb musculature. Four months after the operation the chronaxie values for the gastrocnemii (sciatic nerve stimulation) were determined under amytal anæsthesia, and were found to be 0·02μF (right), 0·03μF (left). At this time the weight of the animal was 8550 g. and the measurements of the limbs showed no appreciable differences between the two sides.

Effects produced by removal of the lower lumbar and upper sacral ganglia.

There are only brief references in the literature to the effects produced by interference with the post-ganglionic path. Boshamer(6) compared the effects produced by section of the pre-ganglionic and post-ganglionic paths in the frog. Employing the method of vascular perfusion and determining the outflow, he found that pre-ganglionic interruption on the one side, and post-ganglionic on the other produced the same degree of vaso-dilatation. If the post-ganglionic rami were cut shortly after the pre-ganglionic, no further dilatation was observed, but if a day were allowed to elapse, the vaso-dilatation produced by the pre-ganglionic section having passed off, the subsequent section of the post-ganglionic rami produced a renewed dilatation. Also the recovery from the pre-ganglionic section occurred within a day, while the dilatation after post-ganglionic section remained for 3 or 4 days.

As it is much more important to acquire some information regarding peripheral vascular adjustments after post-ganglionic interruption in the mammal, the operation was carried out in the dog.

4. Large collie. 16 kg. The left sympathetic cord was removed on December 16th, 1929, from just below the fourth lumbar ganglion to a level beyond the third sacral ganglion (limits confirmed by post-mortem). The animal recovered rapidly and, apart from a transient diarrhœa, remained apparently normal. No evidence of any disturbance as regards gait or tendency to muscular fatigue after very strenuous exercise could be observed on the operated side. The digital temperatures of the right and left feet just before the operation were 29·0° and 28·9°, and immediately after the operation 29·2° and 29·7° respectively. It is advisable in this case to give in tabular form the temperatures subsequently observed on the two sides (Table III). The digital temperatures alone are given.

TABLE III.

Days after operation	Room temperature	Right foot	Left foot
1	12·0°	28·3°	32·0°
2	11·5°	28·0°	32·5°
3	11·8°	27·8°	32·2°
4	12·3°	16·0°	29·7°
		19·9°	19·8°
		(Fore paws)	
7	12·0°	16·5°	28·9°
		16·8°	17·1°
		(Fore paws)	
8	11·0°	16·0°	24·4°
12		14·8°	22·2°
		16·7°	17·0°
		(Fore paws)	
14	12·0°	18·5°	24·0°
16	11·3°	29·3°	31·4°
18	11·2°	26·3°	25·9°
21	12·0°	30·5°	31·6°
22	12·5°	30·0°	29·3°
25	11·0°	28·2°	29·9°
31	10·2°	21·5°	29·8°
		24·2°	24·3°
		(Fore paws)	

It is evident that the cutaneous vascular disturbances in this dog differ from those observed in the preceding ones. For 3 days after the operation the average temperature difference between the two paws was 4·5°, and on the sympathectomized side the temperature remained about the same high level. From the fourth to the fourteenth day the average temperature difference was 9·3°, and this was due to the extremely low temperatures on the normal side, as on the operated side the temperatures were also below those observed during the first 3 days. The extremely low temperature on the normal side was not confined to the hind paw, as it was also observed, although to a lesser degree, in the fore paws. Removal of the sacral ganglia on one side certainly interfered with the corresponding part on the other side. Evidently a reflex vaso-constriction of an exaggerated type was produced in peripheral parts. The right thigh and upper fore limb temperatures were not reduced. The marked difference between the upper and lower parts of the limbs could readily be appreciated by running the hand down the limb, the extreme coldness of the feet being very striking.

The effects of 10-minute periods of rapid stepping exercise in the treadwheel on the vascular adjustments were frequently studied, and a few examples of the results obtained may be given. The digital temperatures were taken before and 5 minutes after the exercise.

TABLE IV.

Days after operation	Before exercise		5 min. after exercise	
	Right	Left	Right	Left
7	16·5°	28·9°	21·0°	28·9°
14	18·5°	24·0°	21·2°	23·7°
18	26·3°	25·9°	28·0°	26·2°
21	30·5°	31·6°	32·9°	31·3°

The rapid stepping exercise was followed by a rise in temperature on the normal side, while on the operated side the temperatures remained almost unchanged. The variability of cutaneous temperatures on the normal side and its marked absence on the sympathectomized side was evident, even without exercise from the third to the fifth week after the operation.

Even when the temperatures on the two sides were approximately the same, the difference in vascular adjustment could be readily demonstrated by the brief application of cooling pads to the feet. For example, on the twenty-second day after the operation when the right and left foot temperatures were 30·0° and 29·3° respectively, a brief exposure to cold (cooling lead-foil cap applied to feet for 1 minute) showed the following changes in cutaneous temperatures on the two sides.

Minutes after cooling	Right	Left	Minutes after cooling	Right	Left
1	26·1°	26·8°	4	26·2°	29·2°
3	26·2°	28·7°	9	26·4°	29·3°

The absence of the normal reflex vaso-constriction on the sympathectomized side after cooling could readily be shown in a variety of ways.

On the twenty-sixth day after operation, the feet were warmed at 43° (dry heat) for 4 minutes and then exposed to the cold air of the basement room (temp. 11·5°), and subsequently the dog was kept standing on the cold stone floor of an open corridor. The temperature changes were as follows.

Original temperature	Right	Left
After 4 min. at 43°	30·2°	29·6°
1 min. later	29·5°	30·8°
2 ,,	29·0°	31·0°
4 ,,	30·2°	31·7°
5 ,,	30·3°	31·5°
2 min. on cold floor		
1 min. later	28·1°	30·7°
3 ,,	28·6°	31·0°
4 ,,	28·5°	31·2°
5 ,,	29·1°	31·2°

Limb musculature. Measurements of the limbs 1 month after the operation showed no appreciable difference between the two sides. After half an hour's very active exercise in the treadwheel, the normal and operated sides showed no difference as regards degree of fatigue.

On passive flexion of the knee joint, however, there was at all times to be observed on the sympathectomized side a diminution in the resistance offered by the quadriceps femoris, and after the elicitation of the knee jerk there was apparently a more rapid diminution in the tone of the muscle.

The chronaxie values for direct stimulation of the gastrocnemii ranged from 0·04μF to 0·08μF on the right, and from 0·02μF to 0·05μF on the left side, that is to say, no significant difference.

A brief reference may be made to another dog, which showed for many months after the operation a very distinct difference in the cutaneous temperatures after exercise.

5. Dog. 18 kg. Left lumbar cord removed on November 16th, 1928. For rather more than a month after the operation the temperature of the left foot was 1°–3° higher than that of the right. On the dates mentioned below there was but little difference between the two sides when the animal was resting. The dog was given half an hour's outdoor exercise, and 5 minutes later, after being brought back to the laboratory, the cutaneous temperatures were the following. The effects produced are typical for sympathectomized animals during the winter months.

Date	Right	Left	Date	Right	Left
10. i. 29	28·5°	33·3°	30. i. 29	28·9°	35·3°
14. i. 29	26·9°	35·0°	9. ii. 29	30·0°	32·6°
21. i. 29	23.9°	32·0°	13. ii. 29	20·4°	33·3°

In this animal no differences as regards the hind limb musculature were detectable.

Summary.

1. After unilateral sympathectomy in dogs in which the interruption was mainly a pre-ganglionic one, the cutaneous digital temperatures in the resting animal were 1° to 4° higher on the operated side than on the normal one for at least a fortnight after the operation.

2. At later periods, the absence or diminution of cutaneous vascular adjustments on the operated side could be shown in a variety of ways, for example, during a post-exercise period on exposure to cold, or during a cooling period following exposure to heat or by the application of cooling pads to the feet.

3. After removal of the three upper sacral ganglia in addition to the lower lumbar ones, the usual rise in temperature on the operated side was observed during the first 3 or 4 days. From the fourth to the fourteenth day after the operation there was a much greater difference in temperature than was observed in the other dogs, mainly due to the extreme vaso-constriction on the normal side. Subsequently the difference between the two sides became less marked, but evidence of disturbance in the cutaneous vascular adjustments could readily be shown by the methods previously described.

4. The limb development, osseous, muscular and cutaneous, in puppies was not interfered with by the operation. In one puppy the increase in weight during a 5-month period after the operation was from 1500 to 9000 g.

5. Apart from an appreciable loss in tone of the quadriceps femoris after the post-ganglionic sympathectomy, no definite disturbance in the limb muscles, either in the resting or actively exercised states, could be detected. The chronaxie values of the muscles were not appreciably altered in any one direction by removal of their sympathetic control.

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THE EFFECTS PRODUCED BY UNILATERAL LUMBAR SYMPATHECTOMY.

II. Muscle metabolism.

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Although a certain amount of information is available regarding the metabolism of skeletal muscle after complete denervation, there is comparatively little obtainable dealing with the effects produced by sympathectomy, and such work as has appeared deals with the amphibian musculature. Büttner(1) refers to an increase in glycogen and lactic acid 1 to 2 days after unilateral section of the rami of the seventh to the tenth spinal roots. He found no alteration in the ammonia of the resting muscles on the operated side, although a rise did occur in the traumatic and stimulated values. Hoffmann and Wertheimer(2) found, after a similar operation in April frogs, no appreciable difference in the glycogen of the resting muscles on the two sides. If, however, the frogs were strychninized, the glycogen in the muscles on the operated side was higher. They found that summer frogs on exposure to heat, and also after adrenaline injections, lost less glycogen on the sympathectomized side. The onset of fatigue was delayed on the side deprived of sympathetic control. These results are rather difficult to harmonize with the experimental findings of Orbeli(3), who found that sympathetic stimulation, following fatigue of the gastrocnemius produced by excitation of the ventral roots of the eighth and ninth spinal nerves, increased the contractions resulting from indirect but not from direct stimulation. It appeared as if the sympathetic influence were exerted through the somatic neuro-muscular terminals. The accumulated evidence of recent years has all gone to show that, at least so far as the dog is concerned, there is very slight, if any, alteration in the general condition of the limb muscles after sympathectomy. As the disturbances which follow this operation mainly affect vascular control, it appears probable that any alterations in the metabolism of muscle may be due primarily

to changes in the permeability of the capillaries or of the striated muscle fibres, so that solvent and solute exchange may be affected. Such changes may, however, only be transient, the accompaniments of variations in functional activity, so that the results obtained from the analysis of sympathectomized muscle may be variable and difficult to interpret. Evidence of an increase in muscle permeability after sympathectomy, based upon results obtained by injection of colloidal dyes, has been brought forward recently by Gabbe(4).

In this communication attention will be specially directed to the water, glycogen, lactic acid, phosphate and creatine values, and also to the reaction of the muscles. As regards the distribution of the various forms of phosphate and the ammonia, it was evident, from the analyses which were made, that variations in these were more likely to be due to extraneous factors rather than to the operative interference.

The animals after the operation were examined for cutaneous temperature changes, etc. (see Part I), and subsequently the muscles were analysed. In studying the effects of sympathectomy on muscle metabolism the animals will be taken in three groups:

(*a*) Those examined from 4 days up to 6 weeks after the operation.

(*b*) Those examined at later periods (3 to 5 months).

(*c*) Those in which the effects of stimulation or exercise of the muscles of the two sides may be compared.

Methods of analyses.

The animals, under amytal anæsthesia, were bled. The muscles, partly frozen *in situ* by a mixture of carbonic acid snow and ethyl chloride or acetone, were then removed, frozen completely, minced, and the material used for analysis.

1. *Water content.* Approximately 1 g. portions were weighed between clamped watch glasses and then dried to constant weight at 110°–115° C.

2. *Glycogen* was determined by Pflüger's method, the glycogen precipitates being hydrolysed in the usual way, and the sugar determined by a micro-modification of Bertrand's method.

3. *Lactic acid* was estimated by the Friedemann, Cotonio and Schaffer method(5).

4. *Creatine* was extracted in the usual way from the minced muscle, the extracts deproteinized and, after transformation into creatinine by heating at 65° in normal HCl for 48 hours, the usual colorimetric determination was made.

5. *Phosphate.* As a rule the amounts of the phosphate in the various

combinations were determined but, for the reason already given, usually only the total inorganic phosphate, that is, the free plus the labile (phosphagen), will be referred to. The free and labile phosphate fractions were frequently determined both by the direct and indirect methods of Fiske and Subbarow(6). The values obtained by the two methods were found to agree closely, but in our hands the phosphagen values were always lower than those usually given by others. The increments in orthophosphate obtained by brief (pyrophosphate fraction) and more prolonged acid hydrolysis were determined in a few cases. The "total inorganic phosphate" value in the tables refers to the sum of the free and labile phosphate, while the "total hydrolysable" includes all the orthophosphate, over and above this value, which has been set free by acid hydrolysis, and therefore includes the pyrophosphate (Lohmann(7)). The creatine-phosphate or phosphagen fraction ranged from 30–60 p.c. of the total inorganic value. The amount of the phosphagen fraction in the muscles on the operated side appeared to be approximately the same as in the control muscles.

(*a*) *Muscles examined from 4 days up to 6 weeks after the operation.*

A. Puppy (6 months), 4 days after the operation.

B. Puppy (6 months), 6 days after the operation.

		(1)	(2)	(3) Phosphate (mol.)		(4)	(5)	(6)
		pH	Water (p.c.)	Total inorganic (*α*)	Total hydrolysable (*β*)	Creatine (mol.)	Glycogen (p.c.)	Lactic acid (p.c.)
A.	N.	6·95	76·3	0·021	—	0·025	1·49	0·088
	S.	7·12	77·3	0·020	—	0·025	1·59	0·067
B.	N.	6·87	77·3	0·0203	0·0147	0·023	0·41	0·102
	S.	6·90	77·8	0·0180	0·0120	0·027	0·32	0·091

N. = normal. S. = sympathectomized muscle.
(Biceps femoris in each case.)

C. A slightly older puppy was examined 6 weeks after the operation. In this case the sartorius (a) and the biceps femoris (b) were both taken for analysis.

		(1)	(2)	(3)			(4)	(5)	(6)	NH_3 (mg. p.c.)
				(*α*)	(*β*)	(*γ**)				
C. N.	(a)	7·05	73·4	0·0163	0·0133	0·0063	—	0·89	0·050	—
S.	(a)	7·07	73·6	0·0197	0·0153	0·012	—	0·88	0·029	—
N.	(b)	—	72·5	0·023	0·0168	0·0098	0·032	0·86	0·049	0·9
S.	(b)	—	72·7	0·023	0·0150	0·0130	0·026	0·94	0·023	0·5

* Pyrophosphate.

It is evident that in these animals there are only slight differences between the two sides. In the sympathectomized muscle, however, the following changes are to be observed:

(1) A rise in the water content.

(2) A slight swing in reaction to the alkaline side.

(3) A lower lactic acid value.

The glycogen shows no appreciable difference, and it is significant that in these animals which have not been specially exercised the glycogen on the two sides may be established, either at a similar high or low level, irrespective of the presence or absence of a sympathetic control. Neither the total phosphate nor the creatine showed in these three animals any significant alteration in one direction.

(*b*) *Animals examined at later periods.*

A. The results obtained in the case of a fully grown dog (16 kg.), examined 3 months after operation, will be given in the first place. The muscle examined was the biceps femoris on each side.

	(1)	(2)	(3) (a)	(4)	(5)	(6)
N.	6·98	72·9	0·0215	0·028	0·356	0·073
S.	6·97	73·1	0·0245	0·030	0·343	0·087

Two puppies were examined 5 months after operation, one (*B*) a very young puppy (4 weeks old at the time of operation), the other (*C*) slightly older (3 months).

		(1)	(2)	(3)		(4)	(5)	(6)
				(α)	(β)			
B. N.	(a)	—	—	0·0226	—	—	0·58	—
	(b)	6·99	75·8	0·0253	—	0·030	0·57	0·039
S.	(a)	—	—	0·0249	—	—	—	—
	(b)	6·98	75·4	0·0276	—	0·030	0·52	0·042
C. N.	(a)	7·04	74·46	0·0180	0·0114	—	0·56	—
	(b)	—	74·48	—	—	0·029	0·88	0·026
S.	(a)	7·08	75·92	0·0196	0·0135	—	0·76	—
	(b)	—	77·01	—	—	0·029	1·09	0·027

(a) = sartorius. (b) = biceps femoris.

Apart from a slight rise in the total inorganic phosphate on the operated side, there is no significant constant difference to be observed in the three animals. In the third animal there was an increase in the water and glycogen on the sympathectomized side, both in the sartorius and in the biceps. From the results obtained in the case of these six animals it became evident that there were no significant differences

uniformly to be observed between the muscles of the two sides. The water content of the sympathectomized muscle was usually slightly higher, and occasionally the glycogen also was slightly higher.

There remained, however, the possibility that excitation of the muscles, either by strychnine or strenuous exercise or electrical stimulation, might bring out some difference in the behaviour of the muscles of the two sides.

(*c*) *Stimulated or exercised control and sympathectomized muscle.*

The effects produced by strychnine were first examined. Injections were given in very small doses over a period of 3 hours until convulsions appeared. The extensor spasms occurred at the same time, and apparently to the same extent on the two sides. As soon as the convulsions appeared, chloroform was rapidly given, the animal bled and the muscle (quadriceps femoris) removed on each side. The results were:

	(1)	(2)	(3) (a)	(5)	(6)
N.	6·63	77·2	0·0246	0·161	0·185
S.	6·90	76·5	0·0257	0·187	0·146

The muscle reaction was much more acid and the water content and the lactic acid higher on the normal side, while the glycogen and the total inorganic phosphate were higher on the operated side.

In another animal the effects of periods of prolonged exercise were tested.

A well-grown puppy, 5900 g., 4 months old, received for the first 2 months after the operation the usual moderate outdoor exercise. Subsequently it received daily 1 hour's very active exercise in a heavy treadwheel during 10-minute periods. The stepping rate in the wheel was a very rapid one, 120–140 per minute. Four months after the operation the muscles were examined on each side.

		(1)	(2)	(3) (α)	(3) (β)	(4)	(5)	(6)
N.	(a)	6·97	75·3	0·0214	0·0264	0·040	0·69	0·025
	(b)	—	75·2	0·0290	0·0250	—	0·61	0·013
S.	(a)	7·07	73·8	0·0205	0·0215	0·041	0·71	0·019
	(b)	—	75·0	0·0270	0·0300	—	0·61	0·017

Apart from the slightly more alkaline reaction on the operated side, there was no appreciable difference in the amounts of the various constituents. The muscles were very rapidly frozen and examined, so that the lactic acid values on both sides are extremely low. The amounts of glycogen

stored by the muscles on the two sides are practically identical. The slight differences in the phosphate are not constant in the two muscles examined, so that evidently the operation has not interfered with the phosphate metabolism.

Another animal exercised in the same way showed also no appreciable differences between the muscles of the two sides, and the analyses therefore need not be given.

In the last animal, which will now be dealt with, the effects of stimulation and recovery after stimulation compared with the resting condition were studied on the control and the operated side. The operation differed from the usual one, in that the upper sacral ganglia were removed along with the lower lumbar ganglia.

Collie. 17 kg. The muscles were analysed rather more than 5 weeks after the operation. The sartorius on each side was stimulated directly for 5 minutes (five induction stimuli per second) and frozen *in situ* while contracting, the circulation not being interfered with during the stimulation period. The gastrocnemius on each side was stimulated in the same way, and about 5 minutes after the stimulation had ceased the muscles were frozen and removed. The biceps femoris in the resting state was also taken from each side. The results obtained from the analyses of the muscles on the control and on the operated side will be given in full.

(i) *The resting muscle (biceps femoris).*

(1)	(2)	(3)	(4) Phosphates (mol.)			(5)	(6)	(7)
Water (p.c.)	Solids (p.c.)	Ash (p.c.)	Free (α)	Labile (β)	Total hydrolysable (γ)	Creatine (mol.)	Glycogen (p.c.)	Lactic acid (p.c.)
N. 74·07	25·93	1·03	0·0062	0·0088	0·0298	0·024	0·71	0·036
S. 76·71	23·29	1·04	0·0090	0·0050	0·0279	0·022	0·57	0·050

(ii) *The contracting muscle (sartorius).*

(1)	(2)	(3)	(4)			(5)	(6)	(7)
			(α)	(β)	(γ)			
N. 76·47	23·53	1·12	0·0096	0·0047	0·026	—	0·20	0·097
S. 77·15	22·85	0·96	0·0058	0·0072	0·022	—	0·45	0·041

(iii) *The recovering muscle (gastrocnemius).*

(1)	(2)	(3)	(4)		(5)	(6)	(7)
			(α)+(β)	(γ)			
N. 75·28	24·72	1·15	0·0139	0·0217	—	0·56	0·017
S. 75·90	24·10	1·05	0·0133	0·0246	—	0·58	0·031

In each state the muscle on the control may be compared with that on the operated side. It is not permissible to compare the resting, contracting and recovering muscle values with one another as these refer to different muscles.

The resting muscle on the operated side has the higher water and lactic acid content. The creatine, total inorganic and total hydrolysable phosphate, and the glycogen are lower in the fresh sympathectomized muscle, but the amounts present per 100 g. solids are approximately the same on the two sides.

The contracting sympathectomized muscle still shows a slightly higher water content, more than double the glycogen, a distinctly higher phosphagen and less than half the lactic acid value of the control.

The muscle on the operated side during the period of recovery has still slightly the higher water content. The lactic acid is now higher than on the control side, but otherwise there are no appreciable differences between the two types of muscle.

Summary.

1. The limb muscles of the dog on the sympathectomized side when examined in the resting state show no significant differences in composition from those on the normal side, apart from a possible slight increase in alkalinity and in water content. The glycogen storage, the lactic acid formation and the creatine content are not appreciably affected by the operative interference.

2. Under the action of strychnine, the water and lactic acid values are higher on the normal side. The glycogen on the operated side does not fall quite so much as on the control side.

3. Regular periods of active exercise do not give rise to any appreciable or significant differences in the composition of the muscles on the control and operated sides.

4. Electrical stimulation of the muscles on the two sides results in a greater fall in glycogen and increase in lactic acid on the normal than on the sympathectomized side. After a period of recovery, the muscles on the two sides show almost no difference in composition. The changes produced by contraction are apparently better recovered from in the muscles on the control side.

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612.621

THE RELATIVE AMOUNTS OF ŒSTRIN REQUIRED TO PRODUCE THE VARIOUS PHENOMENA OF ŒSTRUS.

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I. Introduction.

At the present time œstrin is tested and standardized almost universally by the vaginal cornification reaction of the rat or mouse. Since the amount required to produce this reaction is not necessarily the same as that required for the other symptoms of œstrus, the unit of œstrin as assayed by this method does not necessarily correspond to the amount liberated by the ovaries of a normal animal in order to cause œstrus. Allen and Doisy(2), who introduced the vaginal smear technique for the assay of œstrin, were able to show that the vaginal and uterine œstrous changes, and sometimes copulation, followed administration of this hormone, but they failed to make any study of the comparative amounts required to produce the various phenomena. Most subsequent authors(4, 5, 10), working apparently with amounts of œstrin similar to those required to induce vaginal cornification, have failed to obtain copulation, while little attention appears to have been paid to the uterine changes in test animals.

The unit of œstrin is now usually defined in this country as the amount required to produce vaginal cornification in 50 p.c. of a group of twenty ovariectomized animals. Since the degree of cornification occurring in many of the 50 p.c. of positive reactions is far less intense than that found in the normal œstrous animal, it might be expected that the unit of œstrin, as at present standardized, would be physiologically a comparatively small amount. It has, in fact, been noted already(3, 9) that the amount of œstrin required for vaginal cornification will cause neither the uterine reaction nor copulation.

The present paper deals with a series of experiments designed to

determine the relative amounts of œstrin required in the ovariectomized mouse to produce vaginal cornification, the uterine changes of œstrus, and copulation.

II. Material and technique.

Œstrin. The initial experiments were all carried out with aqueous solutions of œstrin, prepared from urine of pregnancy by methods elsewhere described by one of us(6). Four different batches were used in all (SB 1, PU 4, PU 4ms and PU 4 H); the details relating to the batches may be found elsewhere. No significant difference could be found in the uterine reaction to these different samples. Since, however, the whole of the results obtained with œstrin from urine appeared somewhat anomalous, a special batch of ovarian œstrin was prepared. As a precaution, ovaries were used which had never contained corpora lutea.

960 g. of immature pig ovaries were minced finely and extracted thoroughly with several successive amounts of 95 p.c. alcohol. The combined alcoholic extracts were concentrated to an aqueous residue, and then diluted with water to a volume of 2 litres. This mixture was acidified with 15 c.c. of 12*N* HCl and then extracted repeatedly with ether. The combined ethereal extracts, after washing thoroughly with water, were evaporated to dryness and saponified by heating to boiling for 20 minutes with 200 c.c. of *N* aqueous KOH. Carbon dioxide was passed into this mixture for about 2½ hours, after which it was extracted thoroughly with ether. The ethereal extract was washed first with dilute HCl and then with water and evaporated to dryness. The unsaponifiable matter obtained in this way was extracted three times with small volumes of 70 p.c. alcohol. The combined 70 p.c. alcohol filtrates were evaporated to dryness and dissolved in 30 c.c. of *N*/20 NaOH. This alkaline solution was carefully neutralized to phenol phthalein with acetic acid, the final product being more in the nature of a stable emulsion than a true solution. 0·6 c.c. of this solution was diluted with water to 10 c.c. and injected into a group of twenty mice in four doses of 0·1 c.c. spread over a period of 36 hours. 50 p.c. of the mice showed a full œstrous response corresponding to a potency of 42 mouse units per 1 c.c. of the undiluted preparation.

Administration of œstrin. In the majority of experiments œstrin was injected as for assay, *i.e.* four injections over 36 hours, but in view of the greater effect produced by increasing the number of injections(7) one series of animals had the dose administered in nineteen injections over 36 hours.

Criteria of œstrus. The uterine changes in the mouse at œstrus have been described by many workers(1, 8), and are very easily recognized. The distention of the uterine lumen due to the accumulation of fluid is quite characteristic. Copulation, of course, is detected by observation of the vaginal plug. Vaginal smears were not made from the mice employed in these experiments—the doses of œstrin used were known to produce vaginal cornification.

Time of autopsy. The attempt was made to kill the animals at the time when the most obvious uterine symptom, *i.e.* distention of the lumen, was at its maximum. In the normal animal this distention is found to be complete if the animal is killed when the vaginal plug is discovered. In the ovariectomized animal injected with water-soluble œstrin at 12-hour intervals over 36 hours, beginning a.m., copulation takes place (if at all) 24–28 hours after the last injection and the plug may be found the following morning. 72 hours after the first injection is thus the optimum time for autopsy.

III. Amount of œstrin required to induce uterine œstrus.

The details of fifty ovariectomized mice injected with œstrin obtained from urine are shown in Table I. They give the extraordinary result that

Table I. Amount of œstrin (four injections over 36 hours) required to induce uterine œstrus.

Type of animal	Source of œstrin	Total œstrin (units)	No. of animals	Time after ovariectomy	Mean diameter of uteri (mm.)	Notes
Normal dioestrus	—	—	—	—	1·70	—
Normal œstrus	—	—	—	—	2·85	—
Ovariectomized	—	—	3	15 wks	0·78–0·91	Uteri atrophic
Ovariectomized and used for routine testing of œstrin	—	—	11	3–21 mths	0·74–1·60	Comparatively little atrophy
„ „	Urine	1–21	24	9–20 „	1·20–2·44	No sign of œstrus in uterus
„ „	„	37–74	5	13–14 „	1·46–1·88	„ „
„ „	„	106–111	4	6–13 „	1·45–2·84	One uterus in pro-œstrus
„ „	„	400	3	6–9 „	2·70–3·56	Complete uterine œstrus
„ „	Ovary	4–17	6	7–9 „	1·30–1·68	No signs of œstrus in uterus
„ „	„	109	5	7–13 „	1·76–3·46	One uterus in œstrus

less than 100 "vaginal cornification" units have no detectable effect on uterine distention, while even at this dosage only one mouse out of four showed the effect. Complete uterine œstrus was, however, induced by 400 units. This result was not due to atrophy of the uterus following ovariectomy, because all the mice had previously been used for routine testing of œstrin, and showed no marked degeneration of the uterus. The anomaly suggested that the material obtained from urine might be different in its physiological action from ovarian œstrin. Ovarian œstrin was therefore tested on eleven mice. Those receiving less than 100 units failed to show any signs of uterine œstrus, while only one of five receiving 109 units showed the complete reaction. The original results were, therefore, not due to the source of the œstrin, and it is evident that very considerable amounts of the hormone are required to induce the uterine changes of œstrus.

IV. Amount of œstrin required to induce copulation.

Copulation tests were carried out on groups of, usually, ten ovariectomized mice. Amounts of œstrin ranging from 25 to 200 units were initially given in four doses over 36 hours. As unsatisfactory results were obtained, similar amounts were subsequently given in nineteen doses over 36 hours, *i.e.* injections every 2 hours. This sub-division of the dose increased the percentage of animals copulating, but even so, not more than half of the animals were induced to copulate. Failure due to the male was guarded against by changing the buck during the critical period. The full results are shown in Table II. It is thus clear that the

Table II. Amount of œstrin (given over 36 hours) required to cause copulation.

Amount of œstrin (units)	No. of injections	No. of mice	No. copulating
25	4	10	0
25	19	10	2
50	4	10	0
50	19	9	2
100	4	10	2
100	19	10	4
200	4	10	4
200	19	9	5

induction of copulation, like that of the uterine reaction, requires a vastly greater dose of œstrin than is necessary to produce vaginal cornification.

V. Discussion.

From the results described above, it would appear that the effective physiological dose of œstrin for a mouse is about 200 units. Some such amount is presumably produced by the normal mouse at each œstrous period. The unit as assayed by the cornification test is thus of little significance from a physiological point of view. The smallness of the amount required to produce the vaginal reaction makes possible, however, the assay of small amounts of œstrin in chemical work and, provided that the cornification unit is not looked upon as the amount required to produce complete œstrus, no useful purpose could be served by using either the uterine reaction or copulation as test-objects in the assay of œstrin.

The apparent largeness of the amounts required to produce complete œstrus leads to two conclusions:

(*a*) That the amount of œstrin found in the urine during pregnancy is not so enormous, physiologically, as has appeared to be the case.

(*b*) That no satisfactory clinical results can be expected with the small amounts so far administered to women[1].

Summary.

The amount of œstrin required to produce copulation and the uterine œstrous changes in 50 p.c. of ovariectomized mice is about 200 units as standardized by the vaginal cornification test. Some implications of this relationship are discussed.

Our thanks are due to the Medical Research Council for grants for material and animal maintenance, and to Prof. J. P. Hill, F.R.S., for histological facilities.

[1] On a weight-for-weight basis a woman would require about 2000 times as much as a mouse. Since an ovariectomized mouse requires 200 units to induce complete œstrus, an ovariectomized woman might be expected to require 400,000 units to induce analogous symptoms. Attempts to give œstrin to women by mouth, by which route 50 to 100 units are required to produce an effect equal to 1 unit given subcutaneously, would thus appear to be fantastic.

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612.741

THE ISOMETRIC RESPONSES OF MAMMALIAN MUSCLES.

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The researches of Hartree and Hill(1), Liddell and Sherrington(2), and Fulton(3) have produced much evidence bearing on the nature of the response of a muscle to a repetitive stimulus, *i.e.* a tetanus. Differences between the tetanic responses of "slow" and "fast" muscles have been described by Ranvier(4) and others(5) in the rabbit, by Fischer(6) in the rat and cat, and more recently by Denny-Brown(7) in various muscles of the cat, particularly soleus and gastrocnemius. During the further investigation of the building of a tetanus in the soleus, gastrocnemius, extensor digitorum longus, and internal rectus (eye) muscles of the cat, it was found that the records were being distorted by the effect of friction at the myograph bearing(8). When this source of error was removed or largely reduced, the record of the isometric twitch of each of these muscles was found to be a smooth curve showing neither "angle" nor "period of rigidity"(9). Such "frictionless" myographs[1] have been used for the experiments described in this paper, and, in so far as the frictional error allows, the results are confirmed by our more numerous experiments with the older instruments.

Method.

After decerebration of the cat the muscle to be used was freed as extensively as possible consistent with an intact nerve and blood supply. The lower limb muscles were stimulated by Sherrington shielded electrodes placed on the peroneal (for extensor digitorum longus) or popliteal (for soleus and gastrocnemius) nerves which were cut centrally; all peripheral branches to other muscles were also cut.

[1] Owing to technical difficulties in dealing with the very fine torsion wire of the eye-muscle myograph, friction is still sufficiently great to produce an "angle" in most records of the contraction of internal rectus.

An adequate fixation of the origin of the muscle has been secured by drills through the bone, fixed at both ends by clamps and uprights to the cast-iron top of the experimental table. Microscopical observations have shown a negligible give of the bone even with contractions of 15 kg. For soleus and gastrocnemius the freed bony insertion of the muscle was attached to the lever-arm of the mirror myograph (frictionless) (8) by a short steel hook at a moderate initial tension. The tendon of extensor digitorum longus was securely tied to a ring through which the hook passed.

The internal rectus of the eye was stimulated by a bipolar electrode on the oculomotor nerve as it passes through to the orbit. Reflexes evoked by escape of current are avoided if the ophthalmic nerves are sectioned in the region of the Gasserian ganglion. Fixation was secured by two clamps to the sides of the skull.

From all muscles the electric responses were led off by Ag : AgCl electrodes to a Cambridge string galvanometer. A Lucas pendulum has been used to stimulate the motor nerve by break induction shocks at various intervals, and the tetanic stimulus has been produced by a neon-tube device (10). In all cases the stimuli have been rather stronger than maximal.

The temperature of the muscle has been observed throughout the experiments, and care has been taken to keep it constant (usually between 34° and 37° C.).

Results.

Now that the "angle" has been shown to be an artifact, it has been necessary to introduce another basis for the measurement of the duration of the isometric twitch of a muscle. The interval between the onset of the electrical response and the attainment of maximum tension has been chosen; henceforth this will be called the "contraction time." The approximate "contraction times" of the muscles used are: soleus, 100σ; medial head of gastrocnemius, 40σ; extensor digitorum longus, 40σ; and internal rectus of the eye, 7½–10σ. As would be expected, the durations to the "angle" found by Denny-Brown (7) for these muscles were usually somewhat longer than the values for "contraction time" given here.

The first results concern the effect of a twitch of a muscle on a second twitch following at various intervals. Analysis reveals that many of the features of the double response have counterparts in the tetanic response of the muscle; this latter forms the second part of the investigation.

Time is shown by vertical lines 0·02σ apart. The records read from left to right.

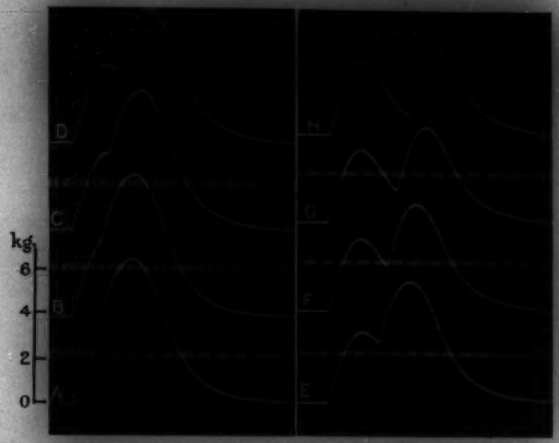


Fig. 5. Mechanical and electrical responses of median head of gastrocnemius to double shocks to peroneal nerve. Temp. 34·5° C. The stimuli were maximal break shocks. Interval between stimuli: *A*, 24σ; *B*, 32σ; *C*, 40σ; *D*, 48σ; *E*, 57σ; *F*, 69σ; *G*, 77σ; *H*, 88σ.

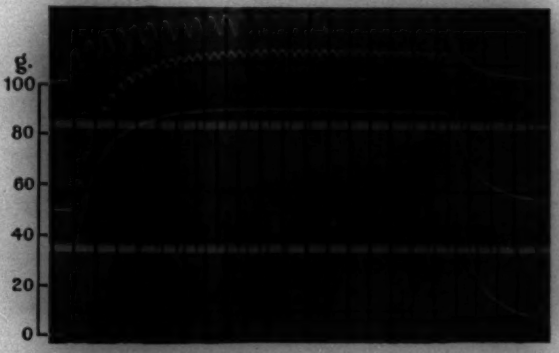


Fig. 6. Mechanical and electrical responses of internal rectus (eye) to tetanic stimulation of n. III. Temp. 36° C. Maximal stimuli from neon-tube device. Rate of stimulation: upper records, 70 shocks a second; middle records, 125 shocks a second; lower records 210 shocks a second. See Fig. 3 *b* for tension-rate curve.

Response of muscle to two stimuli.

Maximum stimuli to the motor nerve have been used to evoke muscular twitches at time intervals, varying from a period just longer than the relative refractory period to one where the second twitch was almost unaffected by the first. Pl. I, fig. 5, shows a series of photographs of double twitches of median head of gastrocnemius. The maximum tension and the "contraction time" of the second response (from the second action-current to the point of maximum tension) give an indication of the alteration of the muscular response as the interval between the two stimuli is increased. Curves with the stimulus interval as abscissæ and both *A* the maximum tension and *B* the "contraction time" of the second response as ordinates are shown in Figs. 1 and 2 for soleus and extensor digitorum longus respectively. Curve *C* shows the single twitch drawn to the same tension time scale; subtraction[1] of it from curve *A* gives curve *D* which represents, for each interval, the corresponding total tension addition from the beginning of the second mechanical response. This tension addition is produced by the second contraction plus any active contractile stress remaining from the first response.

Despite a considerable difference in the time scale, a fairly close resemblance is apparent between Figs. 1 and 2. In both the tension of the double response reaches a maximum with the shortest stimulus interval; however, the ratio to the single twitch tension is 2·2 for extensor digitorum longus and only 1·9 for soleus. Curve *D* reaches a minimum (considerably less than a single twitch tension) at an interval just greater than the "contraction time"; with increasing intervals it slowly returns to the single twitch tension. The early maximum of curve *D* is due to the enhancement of the second response by a considerable amount of active contractile stress of the first response. The gradual increase at intervals beyond the minimum can be explained as the passing off of some influence due to the first response. Certainly for soleus this influence is not entirely the tension remainder of the first response, which for long intervals is less than the tension deficiency of the second response (Fig. 1).

At the shortest stimulus interval the "contraction time" of the second response (curve *B*, Figs. 1 and 2) is considerably longer than

[1] By means of this subtraction it is not intended to make any correction for the effect of the first response on the summated contraction. Any allowance for the influence of one response on another is misleading, as the factors involved are but vaguely understood and certainly not quantitatively estimated

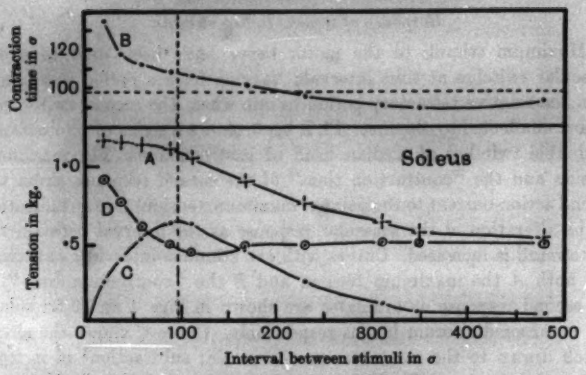


Fig. 1. Curves taken from responses of soleus to double shocks to popliteal nerve. *A*. Maximum tension of double responses. *B*. "Contraction time" of second responses. *C*. Tension of the first response at the beginning of the second response. *D*. The total tension addition from the beginning of the second mechanical response; obtained by subtracting the tensions of curve *C* from those of *A*. The dotted lines show the "contraction time" of a twitch.

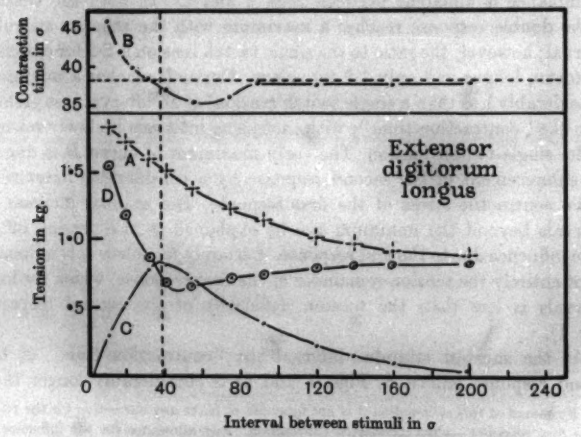


Fig. 2. Curves taken from responses of extensor digitorum longus to double shocks to peroneal nerve. Lettering as for soleus.

that of a twitch. With increasing intervals it gradually diminishes to a value less than a normal twitch "contraction time," and then slowly increases. The return is much slower for soleus, even when calculated on the basis of its longer "contraction time." Gasser and Hill (11), p. 429) have pointed out that, in the isometric twitch, the maximum tension is reached when the external tension development (as recorded by a myograph) has increased to equal the declining internal contractile stress of the muscle. Now, at the moment of maximum tension in the double twitch and still more so in the tetanus, the internal contractile stress is greater than it is for a single response, for, at this instant, it equals the maximum tension which is greater for the repetitive responses. Therefore, in these, the moment of maximum tension indicates an earlier phase of the declining internal contractile stress, and so their "contraction times" are not comparable to the "contraction time" of a twitch, and the marked shortening with certain response intervals must at least be partly explicable in this way.

Gastrocnemius closely resembles extensor digitorum longus, but the ratio of the tension of the maximum double response to that of a single twitch is only 1·9, and the absolute tension values are much greater (see Table I).

TABLE I.

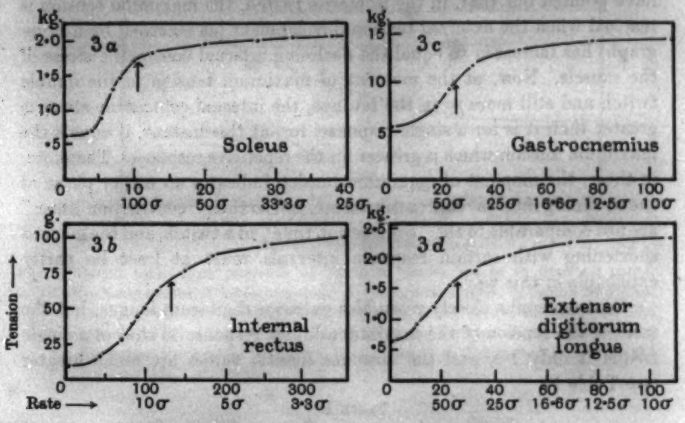
Muscle	Twitch tension (kg.)	(Tension double response) ÷ (Tension single response)	Tetanus tension (kg.)	(Tetanus tension) ÷ (Twitch tension)	"Contraction time" of twitch	Rate of stimulus having intervals = "contraction time" = R (per sec.)	Tension of tetanus at rate of stimulus = R (kg.)	(Tension at rate = R) ÷ (Max. tetanus tension)	Rate of stimulus necessary for complete mechanical fusion (per sec.)
Soleus	·65	1·9	2·44	3·8	100σ	10	1·85	0·76	—
	·65	1·9	2·10	3·26	94σ	10·6	1·73	0·82	33
	·875	—	2·77	3·26	120σ	8·3	2·16	0·78	31
Gastrocnemius	4·64	1·94	14·58	3·3	39σ	25·6	10·1	0·685	100
Extensor digitorum longus	·63	3·3*	3·12	4·95*	$23·4\sigma$	42·7	2·40	0·77	115
	·65	2·2	2·35	3·76	$38·2\sigma$	26·2	1·65	0·74	108
Internal rectus	·0093	>3	0·100	10·7	$7·5\sigma$	133	0·070	0·70	350

* These values are high owing to the rather low initial tension used throughout this experiment.

The responses from the internal rectus of the eye correspond to those from the other muscles if the very short "contraction time" be allowed for; however, the ratio of the tension of the maximum double response to that of a single twitch is greater than 3. When the "contraction time" was $9·1\sigma$, the second twitch was unaffected by the first at an interval of 24σ. The brief "contraction time" is thus associated with a corresponding speed in all the phases of the response.

Response of muscle to repetitive stimuli.

Pls. I and II, figs. 6, 7, and 8, show the responses of soleus, gastrocnemius, and internal rectus to repetitive stimuli of varying rates. In Fig. 3



Rate, stimuli per sec.; and interval between shocks, σ

Fig. 3. Curves showing the effect of rate of stimulation on tetanus tension for the four muscles studied. The values in sigmata under the rates give the interval between stimuli at those rates. The arrow in each case is placed at the interval which equals the "contraction time" of a single response.

measurements from records of this type are plotted with the maximum tension development as ordinates and, as abscissæ, the rate of stimulus rhythm (as shown by the electrical responses). The curves are similar in shape, but, except for gastrocnemius and extensor digitorum longus, corresponding points differ widely in their respective rates of stimulus. If, however, the twitch "contraction time" is used as the standard for time measurement, a very good agreement is found. Thus in Fig. 3 the arrow shows the frequency of the rhythm where each successive stimulus is separated by the "contraction time" characteristic of that particular muscle. At slow rates of stimulus successive twitches have no influence on one another and so the maximum tension of a series is equal to the twitch tension. With quicker rhythms the tension progressively mounts until an undulatory plateau is reached (Pls. I and II, figs. 6, 7, and 8). This is the region of the steep part of the S-shaped curves shown in Fig. 3.

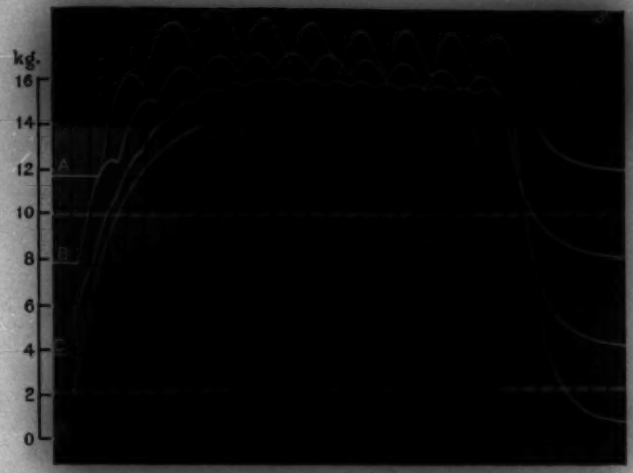


Fig. 7. Mechanical and electrical responses of median head of gastrocnemius to tetanic stimulation of popliteal nerve. Temp. 33° C. Rate of stimulation: record *A*, 19 shocks a second; record *B*, 23·5 shocks a second; record *C*, 35 shocks a second; record *D*, 115 shocks a second. See Fig. 3 *c* for tension-rate curve.

Fig. 8. Mechanical and electrical responses of soleus to tetanic stimulation of popliteal nerve. Temp. 33° C. Rate of stimulation: upper record, 5·3 shocks a second; middle record, 7 shocks a second; lower record, 23 shocks a second. See Fig. 3*a* for tension-rate curve.

Further increases in the stimulus rate produce a more complete fusion, until finally all myographic evidence of the repetitive nature of the response ceases. Practically no extra tension is developed by stimulus rates higher than this. The ratio of the tension produced by such a tetanus to the twitch tension varies for the different muscles (see Table I). The stimulus rate necessary for complete fusion is much higher for internal rectus (about 350 per sec.) than for gastrocnemius and extensor digitorum longus (about 100 per sec.), and this in turn is much higher than that for soleus (about 30 per sec.), *i.e.* the stimulus interval of the rhythm necessary for complete fusion is proportional to the respective "contraction times."

The tension of the repetitive response of soleus does not reach a steady plateau, but, as shown in Pl. II, fig. 8, continually increases. Since fatigue would be produced by the long continuation of the stimulus (several seconds) necessary to develop maximum tension, the somewhat lower tension produced by a stimulus duration of just over a second has been used throughout. On the other hand the responses of gastrocnemius to comparatively slow rates of stimulus have declined in tension after

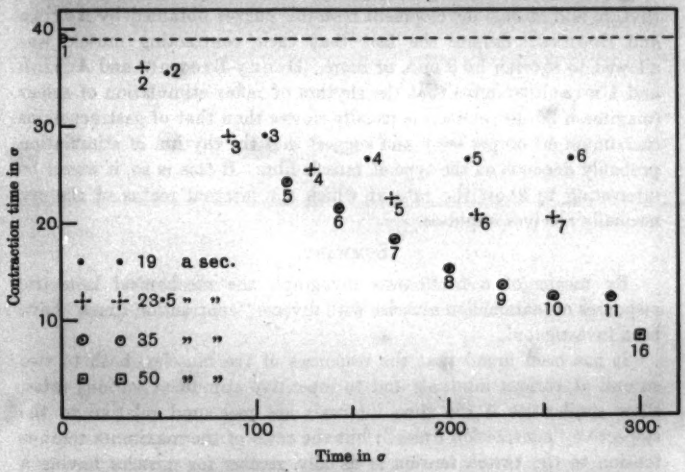


Fig. 4. Median head of gastrocnemius tetanized at various rates through the popliteal nerve. The points in a series show the "contraction times" of successive responses at the rate indicated, and they are plotted at the actual times after the first response. The dotted line shows "contraction time" of a twitch.

an initial increase (Pl. II, fig. 7). This is due to a progressive shortening of the "contraction time" of the successive responses (Fig. 4), which clearly cannot be completely explained by the increased tension. This difference between soleus and gastrocnemius can be correlated with the observed increase in the duration of the second response in soleus at relatively greater intervals than in gastrocnemius (*vide supra*, p. 381). Extensor digitorum longus also shows the decline in tension with comparatively slow rates of stimulus.

Discussion.

Table I summarizes the principal features of the results which have already been discussed. In several respects muscles differing greatly in power and in "contraction time" are closely similar when their responses are measured relative to these respective quantities, *e.g.* the values of the second last column. However it will be noticed that the ratio of the tension development of a repetitive response to that of a single response is greater the shorter the "contraction time."

The S-shaped curves showing the relationship between stimulus rhythm and tension development resemble curves obtained by Adrian and Bronk(12) despite the fact that their contracting muscle was allowed to shorten by 5 mm. or more. Denny-Brown(7) and Adrian and Umrath(13) agree that the rhythm of reflex stimulation of soleus (maximum 30–40 per sec.) is usually slower than that of gastrocnemius (maximum 50–60 per sec.), and suggest that the rhythm of stimulation probably depends on the type of muscle fibre. If this is so, it would be interesting to know the rate at which the internal rectus of the eye normally receives impulses.

Summary.

By means of a frictionless myograph the mechanical isometric responses of mammalian muscles with diverse "contraction times" have been investigated.

It has been found that the responses of the muscles, both to two stimuli at various intervals and to repetitive stimuli at various rates, show similarities if the time intervals are measured relative to the respective "contraction times"; but the ratio of the maximum tetanus tension to the twitch tension is usually greater for muscles having a short "contraction time."

The relation between rate of stimulation and tetanus tension follows an S-shaped curve for all the muscles studied.

We wish to express our thanks to Prof. A. V. Hill for providing the neon-tube stimulating device and to the Christopher Welch Trustees for defraying the cost of photographic material.

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THE PULSE WAVE VELOCITY AND EXTENSIBILITY OF THE BRACHIAL AND RADIAL ARTERY IN MAN.

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In 1923, Bramwell, Hill and McSwiney(1) published the results of investigations on the transmission of the pulse wave by recording simultaneously the carotid and radial pulses. A series of seventy-four observations on normal subjects showed a definite relationship between age and velocity. About the same period, Bazett and Dreyer(2) demonstrated that the pulse wave velocity was lower in the brachial than the radial artery, which suggests a greater extensibility of the central artery. These observers were, however, primarily interested in the relationship of pulse wave velocity to blood-pressure, and endeavoured to correlate their results with the measurements made by Bramwell and Hill(3) on the pulse wave velocity in the isolated human artery with varying internal pressures. No systematic survey was made by Bazett and Dreyer of the velocity of the pulse wave or extensibility of the carotid and peripheral arteries in normal individuals. Hürthle(4), in 1923, showed that the femoral artery is normally less extensible than the carotid artery. This observer investigated the relation between internal pressure and volumetric expansion in living animals by applying a plethysmograph. Using a physical formula, which took into account the diameter and thickness of the vessel wall, Hürthle showed that there was a progressive decrease in the extensibility of the vessels in the arterial path. The present investigation was undertaken to establish the relationship of the pulse wave velocity and extensibility of the brachial and radial arteries with age and, if possible, with the blood-pressure.

Method.

The velocity of the pulse wave was recorded by use of the hot wire sphygmograph as previously described(1). Records were taken from the carotid, brachial and radial arteries of the right arm, with the subject sitting at rest in a chair. A small glass funnel was used to record the

carotid pulse, and a tambour with stud attached, as made by Jacquet, for the radial pulse. For the brachial pulse, a hollow rubber bandage with an effective breadth of 2 cm. was strapped round the arm, just above the elbow, and attached to a Pachon oscillometer. A narrow bandage was chosen, as Bramwell, McDowall and McSwiney(5) had previously shown that, with a sphygomanometer bandage, 11 cm. in width, the velocity under the bandage varied according to the applied pressure. The pressure used in the narrow brachial bandage was never greater than 40 mm. mercury, as it was found that higher applied pressures altered the readings obtained from both the brachial and radial arteries.

To examine the accuracy of the technique, three separate records were made on bromide paper, using a double fibre case in the Einthoven galvanometer. Respiratory movements were recorded simultaneously with the pulse waves, in order that measurements might be made at similar phases of inspiration and expiration(6). The sum of the time intervals of the pulse wave in the brachial and radial artery was found to approximate extremely closely to the time interval obtained by recording the carotid-radial pulse with the brachial bandage removed. Measurements were made in some twenty subjects of different ages, and an average difference of 0·0016 of a second was obtained. As the presence of the brachial bandage, with pressures up to 40 mm. mercury, did not interfere with the results, the three pulse waves were recorded simultaneously in our later experiments. A small Salmanson galvanometer, with two metal strings, was placed in front of the Einthoven string galvanometer, which contained a single glass string.

To obtain the distance traversed by the pulse wave, three measurements were made:

(1) Sterno-calvicular joint to the centre of application of the carotid cup.

(2) Sterno-clavicular joint to the upper border of the hollow rubber bandage applied at the elbow.

(3) Sterno-clavicular joint to the point from which the record from the radial artery was taken.

It is possible, by subtracting the measurements between the sterno-clavicular joint and the carotid cup from the measurements along the subclavian-auxillary trunk, to calculate the length of artery traversed by the pulse wave during the time interval under consideration. Records were made on bromide paper, and the time intervals were measured by the apparatus devised by Elliott. The blood-pressure readings were

taken from the brachial artery by the ausculatory method, immediately after the records were made.

Experimental results.

University students, workmen and school-children were used as subjects, care being taken to exclude those not in good health and incapable of taking muscular exercise. In Table I the pulse wave velocities obtained in fourteen subjects, ages 20 to 21, are given to illustrate the results obtained from the brachial and radial artery.

Table I. Pulse wave velocity.
(Metres per second.)

Age	Brachial	Radial	Whole arm	Blood-pressure
20	4·7	6·1	5·4	110/70
20	4·2	8·1	5·9	106/68
20	5·2	8·2	6·4	112/82
20	5·0	11·3	6·7	102/74
20	5·3	9·1	6·6	100/72
20	4·9	7·6	6·0	98/70
20	4·3	7·3	5·7	98/68
20	4·1	8·7	5·6	112/68
20	4·8	7·2	5·8	120/88
21	4·5	11·0	7·2	118/80
21	4·8	10·4	6·4	108/76
21	4·6	7·2	5·6	108/76
21	4·7	6·7	5·7	132/89
21	5·1	12·7	7·0	134/74
Average	4·7	8·7	6·1	
Minimum	4·1	6·1	5·4	
Maximum	5·3	12·7	7·2	

It will be observed that the pulse wave velocity is lower in the brachial than the radial artery. The measurements obtained from the brachial artery are fairly uniform, but the observations on the radial artery show considerable variation, probably owing to the local conditions of vaso-constriction or dilatation.

The results of our observations on the brachial artery are shown in Fig. 1. The ages of the 103 normal subjects varied from 5 to 80 years, and the pulse wave velocity from 3·1 to 6·6 mm. per sec. Sixty-four observations have been made on subjects under 30 years of age. A smaller number of observations is available over that age, but the results are in good agreement and fall close to the continuation of the mean curve. In Fig. 1 the pulse wave velocity is plotted against age, and a mean curve has been drawn through the observations. Of the 103 observations, 49 fall above, 49 below, and 5 on the mean line. The maximum deviation appears to be 1 m. per sec., and lines are drawn

at that distance above and below the mean line. It will be observed that there is a progressive increase in the velocity of the pulse wave associated

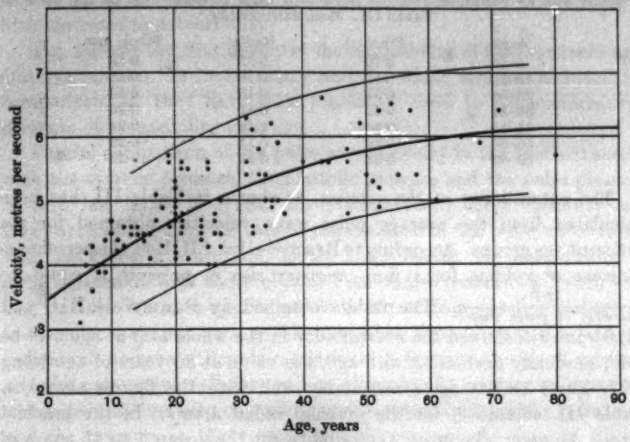

Fig. 1.

with increase in age; this change, however, is more marked in the age groups below 30. Above this age the increase in the pulse wave velocity is more gradual. Although there is a general increase in pulse wave velocity in the radial artery with age, it was found impossible to draw an accurate curve relating velocity to age, owing to the wide variation in the results. Observations made from the carotid and radial pulses showed better agreement, the maximum deviation from the mean line being 1·5 m. per sec. The mean pulse wave velocity in the whole arm represents, to some extent, the average of the velocities in the radial and brachial artery, and it is not surprising, therefore, to find that the results show a moderate degree of uniformity.

TABLE II. Average pulse wave velocity.
(Metres per second.)

Ages	Brachial	Radial	Whole arm
5–15	4·2	6·0	4·9
15–25	4·7	8·3	6·0
25–35	5·0	8·9	6·5
35–45	5·5	10·4	7·0
45–55	5·6	11·4	7·2
55–65	5·6	11·0	7·4
65–80	6·0	11·0	7·5

The average results of the pulse wave velocity obtained in the arteries of the arm in each age group are summarized in Table II.

TABLE III. Mean extensibility.

Ages	Brachial	Radial	Whole arm
5–15	0·70	0·35	0·53
15–25	0·57	0·18	0·35
25–35	0·51	0·15	0·30
35–45	0·42	0·12	0·26
45–55	0·40	0·10	0·24
55–65	0·40	0·11	0·23
65–80	0·38	0·11	0·22

The extensibility of the arteries, as shown in Table III, has been calculated from the average pulse wave velocities obtained for the different age groups. According to Bramwell and Hill (3), the percentage increase of volume for 1 mm. mercury rise in pressure is equal to $\frac{12{\cdot}7}{(\text{velocity of pulse wave})^2}$. The results obtained by Bramwell, Hill and McSwiney (1) showed the extensibility in the whole arm at age 5 to be 0·47, gradually decreasing with age, the value at 80 years of age being 0·17. These results agree reasonably well with the figures shown in Table III, column 3, for the brachial radial artery. In the brachial artery, however, the mean extensibility for the group 5 to 15 years of

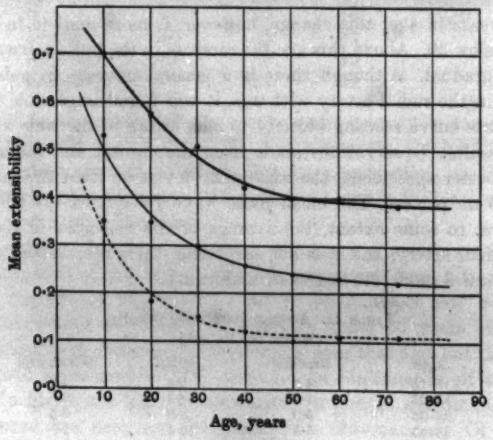

Fig. 2.

——— Brachial. ——— Whole arm. ····· Radial.

age was 0·70, and 0·38 for the group 65 to 80 years of age. It is interesting to note that the extensibility of the brachial artery decreases from 0·70 to 0·42 up to 45 years of age, but with further increase in age there is little decrease in extensibility.

Fig. 2 shows the relationship of the extensibility of the arteries to age. Observations on the radial artery and the whole arm are included for comparison, as they have been previously used to demonstrate the decrease of extensibility with age.

Careful comparison of the pulse wave velocity in the brachial artery with the systolic pressure, the diastolic pressure and the pulse pressure in turn has failed to reveal any definite relationship. Bazett and Dreyer(2) found that the rate of transmission of the pulse wave varies with the blood-pressure and, in the large vessels, the figures were in good agreement with those obtained on excised vessels by Bramwell and Hill(3). We have applied results obtained from the brachial artery in a similar way, but were unable to confirm these observations. In the individual, however, there is a relationship between the blood-pressure and the pulse wave velocity, and an alteration in pressure will produce a change in the velocity depending on the extensibility of the vessel wall and the pressure in the vessel. Measurements of the velocity during inspiration and expiration, and with the limb in different postures(7) demonstrate the effect.

Summary.

By the use of the hot wire sphygmograph the velocity of transmission of the pulse wave in the brachial and radial artery may be measured with accuracy. A series of 103 observations in normal subjects shows that, with the brachial artery, there is a definite relationship between age and velocity. It is further shown that, between 5 and 80 years of age, the arterial extensibility decreases from 0·70 to 0·38. The pulse wave velocity was found to be always lower, and the extensibility higher, in the brachial than in the radial artery. The rate of transmission was much more variable in the peripheral than in the central artery. No relationship between pulse wave velocity and arterial blood-pressure could be established.

The expenses of this research have been defrayed in part by a grant from the Government Grant Committee of the Royal Society.

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TONUS IN MAMMALIAN UNSTRIATED MUSCLE. I.

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Introduction.

Theories accounting for tonus in unstriated muscle may be divided into two classes: (1) those which depend on some novel mechanism, such as a catch mechanism or gelation, and (2) those which are based on an analogy with similar phenomena in other tissues, such as tetanus in striated muscle. The chief evidence justifying the former kind of explanation was derived from experiments which seemed to show that the tonic contraction of plain muscle was associated with little or no increase of oxygen consumption above that of the relaxed muscle. Recently, Bayliss(1) has calculated, however, that the oxygen consumption corresponding to a tetanus in plain muscle would not necessarily be enough to be detected by the methods employed. This is due to the characteristic slowness of the relaxation, which enables a low frequency of stimulation to produce a fused contraction. The speed of relaxation depends on the so-called "viscosity" of the muscle; and since also it has been shown by Gasser and Hill(2) that the viscosity of skeletal muscle is increased during contraction, it seemed likely that a study of the conditions affecting the viscosity of plain muscle would throw light on the problem of tonus.

Analogy with the reflex tonus of skeletal muscle would suggest that a response to stretch might play a part in the tonus of those unstriated muscles which incorporate a local peripheral nerve plexus, and observations on intestinal and other musculature reinforce this belief. Tonus can be demonstrated, however, in isolated muscles which include no nerve cells, and exhibit no obvious response to stretch. If, then, tonus is to be reduced to its simplest terms, it is evident that it should be analysed first in these simpler muscles. With results so obtained it may be hoped that the superposition of a nervous mechanism will not render insoluble the problem of tonus in the more complex muscles. The retractor

penis of the dog was therefore chosen as suitable, since it includes no nerve cells, and it does not appear to be stimulated by stretch. It has the further advantages of having its muscle fibres longitudinally arranged, and usually showing no spontaneous contractions. I am indebted to the courtesy of a number of colleagues who allowed me to remove such muscles from experimental animals which had been used by them for other purposes.

Some mechanical aspects of tonus. The forces with which a contracted muscle resists stretch may be of four kinds: (1) that which is independent of the displacement (comparable with surface tension or weight), (2) that which is a function of the displacement (comparable with elasticity or buoyancy), (3) that which is a function of the velocity of displacement (viscosity), and (4) that which varies with the acceleration. Forces of the last class depend on inertia and will be so small in comparison with the other forces concerned in the slow movements of plain muscle that they can safely be neglected.

The three remaining kinds of force may be related in countless ways, which can be paralleled by the complex networks of resistance, capacity, and inductance studied by the electrical engineer. Although it is now the custom to analyse elaborate mechanical systems by reference to the corresponding electrical models, whose properties are more completely understood, this procedure will not be adopted; it is only mentioned to emphasize that the mechanical models to be described are intended to convey the relations between the tension and length of the whole muscle, and time, in mechanical symbolism, in the same sense as the analysis might have been performed in electrical symbols, or in algebraic symbols. The mechanical model happens to involve the same concepts—force, distance, and time, as are concerned in tonus, but it is only a logical fiction invented in order to simplify the description of the relations between them, and does not aim at representing how the muscle actually produces such relations.

Levin and Wyman(3) examined the effects of stretching skeletal muscles, and found that the changes of tension with time could be represented by two springs arranged in series, the extension of one of which was damped by a vane moving through a viscid liquid as in Fig. 1. The springs obeyed Hooke's Law, and the viscous resistance was proportional to the velocity of the vane at any given moment. Now, although the details are not quite so simple, the qualitative results of stretching unstriated muscle are the same. If the tension in the muscle be recorded, a sudden stretch produces an immediate large increase of

tension (equivalent to stretch of the undamped spring only), and if the muscle is kept at the new length this is followed by a gradual falling off of the tension as in curve *A* (gradual extension of the damped spring allowing the undamped spring to shorten without change of length of the whole system). Conversely, sudden shortening of the muscle produces an immediate large fall of tension followed by a gradual recovery till a new equilibrium is established, as in curve *B*. If, on the other hand, the changes in length of the muscle be recorded, the sudden application of a constant tension results in an immediate stretch (extension of the undamped spring) followed by a gradual further stretch (extension of the damped spring, while the undamped spring remains unaffected) as in curve *C*. In the model, the gradual stretch is an exponential function of time; and under ordinary isotonic conditions of recording the curve becomes a straight line only when it is horizontal. It will be shown that the properties of plain muscle deviate from those of the model in this respect. When the load is suddenly removed from a stretched muscle, the corresponding sudden (undamped) and gradual (damped) portions may be distinguished while it shortens as in curve *D*. These general reactions to stretch and release have been observed in many mammalian plain muscle preparations, such as the guinea-pig's uterus, and the rat's duodenum. In those muscles which normally undergo rhythmic contractions, the reactions are most clearly revealed after the spontaneous activity has been abolished by immersion in solutions of chloral or other suitable narcotics. The same type of reaction to stretch and release was found in Holothurian smooth muscle by Hill(4).

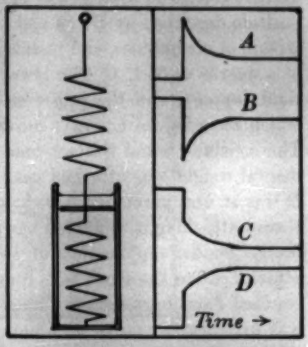

Fig. 1. Viscous-elastic model of Levin and Wyman. The upper curves show the relation between tension and time when the model is suddenly lengthened (*A*), and shortened (*B*). (Increase of tension recorded vertically upwards.) The lower curves show the relation between length and time when an isotonic load is suddenly applied (*C*), and when it is suddenly removed (*D*). (Increase of length recorded vertically downwards.)

It is evident, therefore, that the force maintaining the tonus of plain muscle may be analysed into at least one undamped elastic system, and one viscous-elastic system; and we will consider in turn the variations of the elements of these systems under various conditions.

Experimental methods. The slow time relations which are characteristic of unstriated muscle enable the simplest methods to be employed without undue error. The muscle was immersed in a Ringer's solution of composition described by Burn and Dale(5), which was stirred by a regular stream of air bubbles, and maintained thermostatically within one-tenth of a degree of 36·4° C. The lower end of the muscle was attached to a rigid support, and the upper end to a light aluminium isotonic lever which recorded on a slowly moving drum with a magnification of 2·3. The constant initial isotonic load was about 0·2 g. weight. The experimental weight was attached near the fulcrum of the lever by a thread. It was at first prevented from loading the lever by support from another thread attaching it to a fixed bar placed above and perpendicular to the lever. Sudden application of the load was effected by burning the attachment to the fixed bar. Subsequent sudden release from the load resulted from burning the thread attaching it to the lever. The drum was arranged to move at about 1 cm. per sec. during the first two minutes, in order to secure a sharply defined demarcation between the sudden and gradual phases of the stretch. The later phases of the stretch were recorded with the drum moving more slowly.

The undamped elastic system. The response to sudden stretch of the retractor penis was described in a previous communication ((6), Fig. 5), where it was shown that the immediate tension development due to a series of stretches through equal absolute distances increased with increasing length of a muscle. It may be inferred that the equivalent spring becomes stronger when the muscle is longer. One might expect, and does indeed find, that the application of a series of increasing loads to a muscle would result in extensions which indicate decreasing compliance of the undamped element, as shown in Fig. 2. The sudden shortening which is produced by removing a particular load from the extended muscle is usually less than the sudden lengthening which that load produces in the contracted muscle, as shown in the two pairs of curves (VIII and IX). Two kinds of exceptions to this rule may be mentioned. First, when the muscle is so fully relaxed that the resilience due to the boundary elastic system reinforces that due to the undamped spring, the sudden shortening is abnormally great, as it was when 12½ g. weight was removed in the experiment represented in curve VIII. Secondly, certain muscles consistently show the same or even a greater shortening than the lengthening produced by the same load; and the reactions of one such muscle are represented in curve VII. The conditions producing this anomaly have not been discovered.

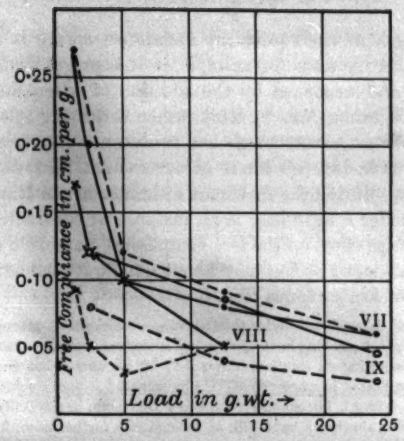

Fig. 2. Variations, with increasing loads, of the compliance of the undamped elastic phase of the stretch (continuous lines) and release (broken lines) of the isolated retractor penis. Each pair of curves corresponds with one of three muscles: Nos. VII, VIII and IX. The curves show increase of the strength of the equivalent spring with increase of load.

Fig. 3. Record of the stretches of a retractor penis due to sudden isotonic loading. (The early portion only is shown.) Load in curve (*a*) 5 g., (*b*) 12·5 g., (*c*) 24 g., (*d*) 12·5 g., (*e*) 24 g., (*f*) 12·5 g. Curves *d* and *e* represent stretches in the presence of adrenaline ($1 : 10^9$). The curves indicate the order of accuracy with which the undamped and damped portions of the stretch may be distinguished. Comparison of *b* with *d*, and of *c* with *e*, shows the effect of adrenaline in weakening the equivalent undamped spring.

The strength of the equivalent undamped spring is affected by a variety of other factors; for example, it is strengthened by a fall in temperature, and weakened by the addition of adrenaline ($1 : 10^6$) to the Ringer's solution (Fig. 3). This rather surprising relation between the free compliance of the muscle and contraction due to a drug cannot be extended to include all kinds of contraction. The development of "Ringer tonus" during the first hour of immersion in Ringer's solution of a muscle under a light load is an instance of contraction associated with the opposite effect on the free compliance, namely, a decrease. The equivalent undamped spring may about double its strength during the development of Ringer tonus (Fig. 6).

"Ringer tonus" is produced under the following conditions. A retractor penis, which may be, for example, 4 cm. long in the living dog, is excised and immediately dropped into cold (17° C. or less) Ringer's solution. After 5 to 10 minutes it has contracted to, say, 2·5 cm. It is then placed in warm (36–37° C.) oxygenated Ringer's solution, with a light isotonic load (*e.g.* 0·20 g.). The muscle relaxes to about 3 cm. in the course of one-quarter of an hour, and then slowly contracts till, at the end of a further hour, its length may be 1·5 cm. This condition of contraction is maintained so long as the muscle survives, and is what I have termed "Ringer tonus." For reference, it may be added that a retractor of the dimensions named would be about 8 cm. long when fully extended, and that the orders of time needed to reach practical equilibrium, when relaxing under an isotonic load of about 10 g., would be one-half an hour if the load were applied when the muscle was first placed in the warm bath, and 5 hours if the load were not applied until Ringer tonus had fully developed. The viscous and elastic properties characteristic of Ringer tonus can be modified by subsequent treatment of the muscle with adrenaline.

The action of adrenaline on the undamped elastic system is shown in Table I, which includes instances where the development of Ringer tonus made it possible to arrange for the initial lengths of the control and drugged muscle not to be widely different.

TABLE I.

		Control		Adrenaline	
Exp.	Load (g. wt.)	Free compliance (cm./g.)	Initial length (cm.)	Free compliance (cm./g.)	Initial length (cm.)
V	10	·040	3·8	·068	4·3
	20	·031	4·1	·063	4·3
VI	12½	·045	2·1	·104	2·2
	24	·048	3·1	·100	2·4

In seven other experiments in which the length of the muscle, under the influence of adrenaline, was shorter than its control length, the immediate compliance in the presence of adrenaline averaged 172, taking the control value as 100. The effect of adrenaline on the sudden release was not so large, the compliance being increased only to 120 (average

of nine experiments), as compared with the release control value of 100. This effect of adrenaline is small or absent, when the load applied is 3 g. or less.

The effects of contraction on the viscous-elastic system. These have been observed under isotonic conditions by measuring the progress of the gradual lengthening or shortening of the muscle, which follows the instantaneous changes produced by sudden application or removal of a load.

The addition of adrenaline ($1 : 10^6$) to the solution in which a retractor is immersed produces a well-marked speeding-up of the relaxation due to a load, as shown in Fig. 4. On the other hand, the muscle

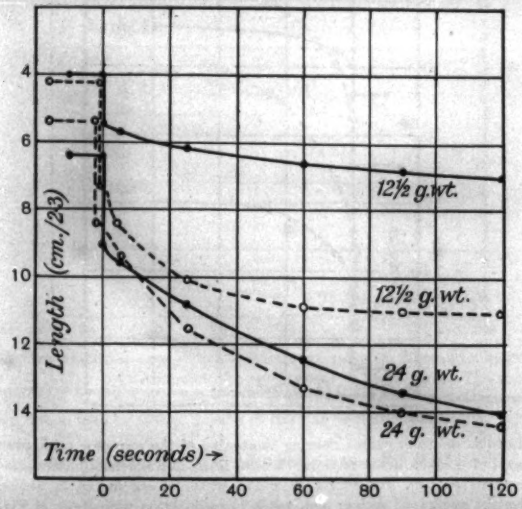

Fig. 4. Isotonic extension-time curves showing the pure elastic and the early part of the viscous-elastic stretch of the retractor penis, in the presence (broken lines) and absence (continuous lines) of adrenaline ($1 : 10^6$). The curves show the effects of adrenaline in weakening the equivalent undamped spring; and, taken in conjunction with those of Fig. 5, they show its effect in reducing the viscosity of the viscous-elastic component.

does not relax so far in the presence of the drug as it does, if left long enough, in its absence. The rapid initial relaxation is therefore not due to a weakening of the equivalent damped spring, which is actually strengthened; but it must be attributed to a reduction in the viscous

resistance. If the relaxation curves be regarded as exponential, as a first approximation, the reduction of "viscosity" due to adrenaline may be calculated to be of the order of twenty-fold. (By "viscosity" is meant the ratio of the viscous resistance in g. wt. to the velocity of the free end of the muscle, in cm. per sec. It is proportional to the product of the time of half-relaxation and the compliance of the equivalent spring.)

The diminution of viscosity due to adrenaline is shown equally well in the release curves (Fig. 5); and the equivalence of the effects of

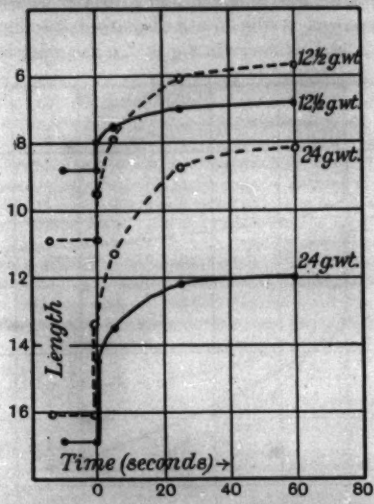

Fig. 5. Isotonic recovery curves showing shortening of the retractor penis after sudden removal of a load, in the presence (broken lines) and absence (continuous lines) of adrenaline ($1 : 10^6$). The curves show the effects of adrenaline in weakening the equivalent undamped spring; and, taken in conjunction with those of Fig. 4, they show its effect in reducing the viscosity of the viscous-elastic component.

adrenaline on the lengthening and shortening of the muscle gives no support to the hypothesis of a polarized mechanical system, such as the "catch mechanism."

In contrast with the hastening action of adrenaline, the changes associated with the development of Ringer tonus are in the opposite sense, as shown in Fig. 6.

Analysis of the properties of the viscous-elastic system. Inspection of

the length-time curves during isotonic stretching of a retractor usually shows at once that there is no simple exponential relation between these

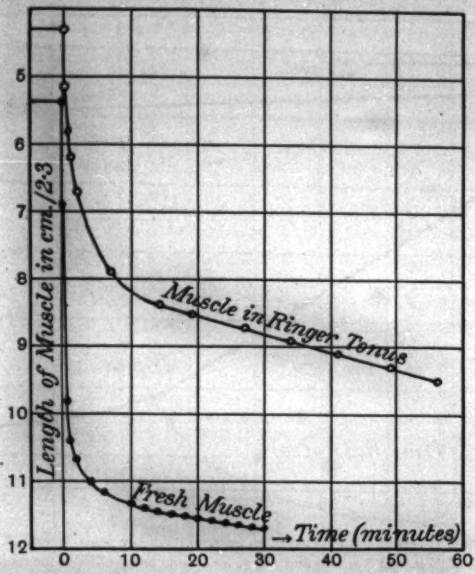

Fig. 6. Isotonic extension-time curves of a retractor penis (1) 20 minutes after removal from a dog, (2) 1 hour later after the development of Ringer tonus. The curves show the influence of the tonic contraction in strengthening the equivalent undamped spring, and in increasing the viscosity of the viscous-elastic component.

two variables. The curves often become practically straight lines, while the slope is still considerable—as illustrated in Fig. 7. The straight portion of the curves represents stretching at a uniform velocity. If the viscosity is constant, the force acting upon the viscous system must therefore also be constant. The damped elastic system has spent its force during the early curved portion of the relaxation, and the muscle then extends like a pure viscous system under constant tension until the boundary region of complete extension is reached. This introduces a new elastic system, which gradually effects an equilibrium length for the given load when the muscle is fully extended, and which persists after death of the muscle.

The disappearance of the elastic component suggests either some nervous inhibitory process due to the prolonged action of the load, and

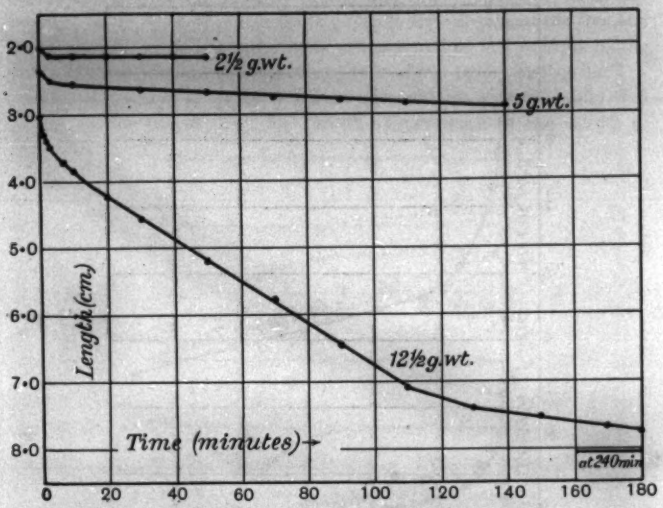

Fig. 7. Isotonic extension-time curves of an isolated retractor penis, stretched successively by loads of 2½ g. wt., 5 g. wt., and 12½ g. wt.—time being allowed for recovery of tone before the application of each. (The undamped stretch is omitted.) The curves show that the viscous-elastic phase is terminated by an inclined straight line, corresponding to a uniform velocity, and attributed to an equivalent pure viscous phase.

analogous with the lengthening reaction of innervated skeletal muscle, or some irreversible change in the surviving tissue. The first is rendered unlikely by the observation that the same kind of discontinuity in the relaxation curve persists in concentrations of chloral hydrate (1 : 500) more than enough to abolish all spontaneous activity in muscles which have shown it. The second suggestion is contradicted by the subsequent properties of the muscle, as illustrated in Fig. 8. This retractor was first stretched by a light load (2½ g.) and released. It was then fully extended with a large load (24 g.) and released. Finally, it was again stretched by the light load (2½ g.) and released. The curves obtained before and after the complete stretch, which included a period of relaxation at uniform velocity, were practically identical, although insufficient time

was allowed for the muscle to shorten quite to its original length. The curve (Fig. 8) showing lengthening of the same muscle when a heavy

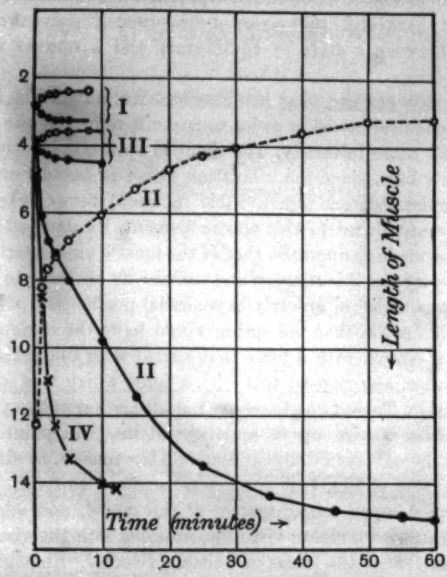

Fig. 8. Isotonic curves showing extension (continuous lines) and recovery (broken lines) of a retractor penis. Curves I = stretch and release with load of 2½ g. Curves II = stretch and release with load 24 g. Curves III correspond with Curves I. Curve IV is the stretch with a load of 24 g. in the presence of adrenaline ($1 : 10^6$). The curves show that a full extension, including a phase of stretch at uniform velocity, involves only reversible processes. (The undamped phase is omitted.)

load (24 g.) was applied in the presence of adrenaline ($1 : 10^6$) is included both to amplify the evidence shown in Fig. 4 by describing the changes during a longer period of time, and to indicate some of the conditions under which no straight portion of the relaxation curve is found. The phase of uniform velocity is abolished when the muscle lengthens far enough during the viscous-elastic phase of retardation to reach the second phase of retardation due to complete extension. This tends to occur with large loads, with low viscosities, and particularly when both these conditions are combined, as in the curve shown. Another class of

conditions which makes the phase of uniform velocity difficult to detect is the combination of small loads with high viscosities, as illustrated in the top curve of Fig. 7. Here the rate of relaxation is so small, after the early part of the stretch, that errors of measurement prevent a confident distinction between a state of equilibrium and a minute velocity of extension.

We may now enquire what modifications will have to be introduced into the mechanical model in order to make it represent the properties of the muscle more faithfully. The damped spring evidently does not obey Hooke's Law, since the relaxation curve is not exponential. If a more complex relation (*e.g.* a cubic function) between tension and extension be attributed to this elastic element, its damped relaxation curve may be made to approach that of the muscle more nearly. But for our present purposes it is simpler, and sufficiently accurate to regard the curve as compounded of an early exponential portion and a later linear portion. This implies that the spring would have the curious property of resisting a stretch with a force that varied with the extension for a certain distance, and beyond this with a force which was independent of the extension. These two phases are not altogether unlike those found when stretching a wire up to and beyond its yield point. In view, however, of the effects of different loads on the muscle, as shown in the lower two curves of Fig. 7, it is more satisfactory to retain the simple spring in the damped elastic system of the model, and adopt a pure viscous system with a higher viscosity, in series with the viscous-elastic system, to introduce the phase of uniform velocity. Analogous phenomena in wires have been represented by a somewhat similar mechanical model by physicists in order to simplify their analysis (7).

The model constructed in this way is illustrated in Fig. 9, accompanied by a curve showing the three phases—pure elastic, viscous-elastic, and pure viscous—of the length-time curve produced by sudden application of a load under isotonic conditions. In this arrangement the stretch due to the pure viscous system is at a uniform rate throughout, and its amount at any instant can be calculated from the velocity shown by the straight portion of the curve. Thus by subtracting the pure viscous stretch at any instant from the total stretch at that instant, the viscous-elastic stretch can be found. The viscous-elastic stretch, derived in this way and plotted against time, would give a simple exponential curve, represented by the line *B*. From this curve the extensibility of the spring and the viscosity can be calculated. If the relaxation curves obtained from muscle are analysed by this method, the derived "viscous-elastic"

curves are approximately exponential in form, and the equivalent spring approximately obeys Hooke's Law.

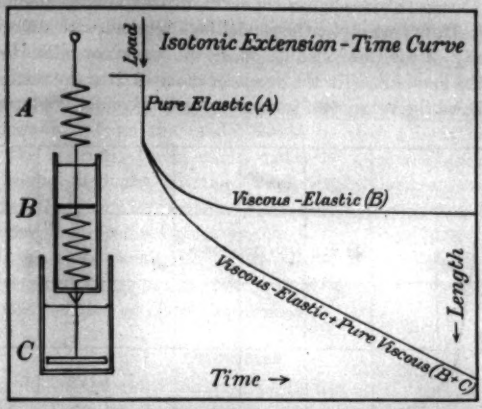

Fig. 9. Triple mechanical model representing the relations between tension, extension, and time, during the stretch or release of a retractor penis. The elastic elements are shown as coil springs, and the viscous elements as vanes moving in viscid liquids. The viscosity of C is much higher than that of B. The diagram of isotonic extension-time curves indicates the contribution of each component of the model: (1) the pure elastic component (A) giving an instantaneous (vertical) response to loading; (2) the viscous-elastic component (B) giving an exponential response, and (3) the pure viscous component (C) giving a linear response. The complete curve ($A+B+C$) represents the response of the whole model.

The development of tonus. When a muscle is released from a load which has stretched it, it shortens to somewhere near its fully contracted length. We have seen that a large part of the stretch could be represented as that of a pure viscous system under the influence of a constant force, presumably equivalent to the isotonic tension. The question arises, what is the restoring force which enables the muscle to shorten through the length corresponding to the non-elastic portion of the stretch? This force is evidently independent of the extension, and resembles surface tension in this respect. The reason why this force does not make the muscle contract up to nothing is suggested by inspection of the fully contracted muscle, which shows parts of the tissue beginning to buckle. Most of the tissue is probably prevented from buckling by the adhesion of adjacent portions, and this introduces a second elastic boundary system corresponding with the completely contracted length of the muscle.

The constant restoring force may be represented in the model (Fig. 9) by adding weight to the damping vane of the pure viscous system. Certain consequences follow from this arrangement, and may be tested. If, for example, the model be stretched with different loads, the velocity of the pure viscous phase will vary not with the absolute value of the load, but with the excess of the load over the restoring force. Fig. 10 shows the velocity of isotonic stretch of a muscle plotted against

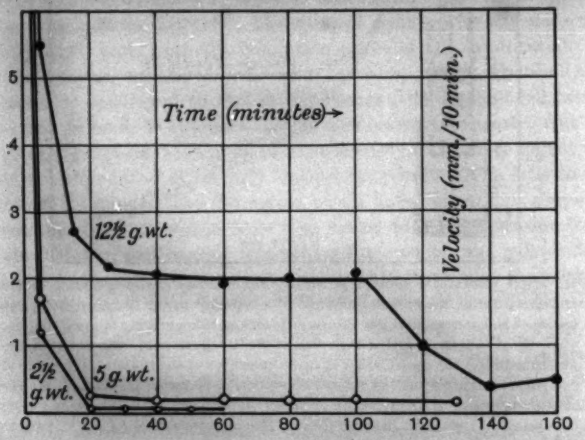

Fig. 10. Velocity-time curves obtained by stretching a retractor penis with a series of constant loads. The curves show (1) the initial exponential phase (viscous-elastic), (2) the phase of uniform velocity (pure viscous), and (3) a terminal viscous-elastic phase when the boundary region of complete extension is approached. A ten-fold increase of the uniform velocity is produced by a two-and-a-half-fold increase of load.

time for a series of loads. Considering the horizontal portions of the curves, which represent the uniform velocity of the pure viscous phase, it will be seen that a load of 12½ g. produced about ten-fold the rate of stretch which was produced by 5 g. This would be intelligible if the restoring force opposing both these loads were about four grams weight, and the excess of the actual load over the restoring force were the effective force opposing the viscous resistance. Another inference from the model would be that loads exerting a force less than the restoring force would have no effect on the pure viscous system, and should therefore produce an exponential relaxation curve, corresponding with the viscous-elastic

system. The whole model should reach equilibrium at a much shorter length than its fully extended length. It is difficult to be sure whether this is true of muscle, owing to the exceedingly slow stretches induced by light loads, for example, by 2½ g. in Fig. 10, and the consequent difficulty of distinguishing between the almost asymptotic limb of an exponential curve, and a minute superimposed uniform velocity. But the evidence available is clearly not in conflict with the inference. Another deduction from the model would be that a light load (just exceeding the restoring force) might suffice to keep a relaxed muscle extended, though it might take indefinitely long to induce a contracted muscle to lengthen very far; and this is in accordance with the experience of many workers on isolated plain muscle.

Straight portions occur also in the release curves (length-time) of the muscles, but they are not so prominent as those in the stretch curves, and have not yet been so closely scrutinized.

Discussion.

The length of the retractor at a particular time can therefore be represented in terms of the reaction of several mechanical systems to the tension. That the effects of small differences in experimental technique should play so prominent a part in producing apparently inconsistent changes in the length of smooth muscle at different times, and in different places, is hardly surprising when it is regarded as a resultant of so many variables. The action of adrenaline on the retractor penis may be quoted as an example. Adrenaline regularly induces contraction. In only two, out of over a hundred muscles to which adrenaline has been applied, has the reaction to the drug been inhibitory. Both these muscles were tonically contracted. Similar ambiguities in the responses of other plain muscles to drugs and nervous stimulation have been recorded. The ambivalent effects of adrenaline described above on the different components of the equivalent mechanical system would readily account for such anomalies.

Two kinds of contraction in the retractor have been studied in the foregoing experiments, namely, the development of Ringer tonus, and the response to adrenaline. Though shortening of the muscle is common to both, the other properties of the muscle which have been examined vary in opposite directions in the two cases. Ringer tonus involves a small tension, and shortening prevented by a load exceeding 3 to 4 g. This tension appears to correspond with the non-elastic restoring force mentioned above, and is therefore largely independent of the extension

of the muscle. Perhaps the most prominent feature of Ringer tonus is the high value of the viscosity which presumably ensures maintenance of a particular length with minimum metabolic expenditure, while opposing a large force to any rapid change of length. It may be convenient to refer to this as the postural contractile mechanism. The response to adrenaline, on the other hand, may involve the development of ten- to twenty-fold the tension, and, as has previously been shown ((6), Fig. 7), the value of this tension varies with the length of the muscle. Associated with this, there is a large reduction in the viscosity which facilitates rapid changes of length. This may be termed the phasic contractile mechanism. Auxiliary evidence favouring the distinction between these mechanisms is derived from observation of spontaneous contractions and the responses to short electrical stimuli occurring during the slow isotonic relaxation under load of a muscle which has developed Ringer tonus. After either of these disturbances, the muscle returns to the length which it would have reached if it had continued in uninterrupted relaxation during the same period of time.

The phasic and postural states may perhaps be regarded as opposite extremes of a continuously variable condition of the muscle—the resting muscle in the living animal probably occupying an intermediate position.

If these mechanisms, found in the isolated retractor penis surviving in Ringer's solution, can also be demonstrated in the musculature of the hollow viscera, the postural system, on the one hand, would be admirably adapted to gripping the contents firmly but not tightly, to showing small changes of tension corresponding with large changes of length so long as these proceeded slowly, and to maintaining its tonus economically. The phasic system, on the other hand, might be regarded as mobilizing the muscle for rapid expulsion of the contents, requiring more force and less viscous resistance for large but transient changes of length—sustained contraction of this kind being uneconomical.

The mechanical analysis of tonus has revealed two boundary elastic systems, corresponding with complete contraction and complete extension; between these limits, the variables concerned are related approximately as in the triple model. Of the relations between the components of the model, only that of the undamped elastic system and the viscosity has been examined under a variety of conditions. The equivalent free spring is strengthened, and the viscosity increased during the development of Ringer tonus or by cooling the muscle. The opposite effects on both systems are produced by adrenaline. The parallelism between these distinct components of the model under a variety of conditions should

be a sufficient warning to beware of an attempt to attribute any anatomical significance to the elements of a mechanical model, which is designed only as a trick to simplify the systematic description of the changes in length, tension, and time, of the entire muscle.

The retractor penis is not unique among plain muscles in exhibiting linear portions in the isotonic stretch and release curves, for similar lengthening and shortening at uniform velocity has been observed in the adductor muscle of *Pecten* (8).

Summary.

1. The nature of the force with which a tonically contracted smooth muscle resists stretch has been analysed by sudden application of various loads under isotonic conditions.

2. Two contractile mechanisms may be distinguished in the isolated retractor penis, which is devoid of nerve cells and exhibits no active response to stretch.

(*a*) The postural contractile mechanism, exemplified in the development of Ringer tonus, is associated with (1) a small tension (*e.g.* 3–4 g. wt.) which may be independent of the length of the muscle within a considerable range, (2) a high viscosity, which results in a large tension being opposed to quick increases of length, and may be expected to enable the muscle to remain contracted at the expense of relatively little metabolic activity, and (3) a relatively rigid undamped elastic component.

(*b*) The phasic contractile mechanism, exemplified in the response to adrenaline, is associated with (1) a relatively large tension (*e.g.* 40–50 g. wt. at the optimum length) which is approximately proportional to the length of the muscle within a considerable range, (2) a low viscosity, which results in only a small tension being opposed to rapid changes of length, and may be expected to involve relatively intense metabolic activity during a sustained contraction, and (3) a relatively extensible undamped elastic component.

3. The relations between tension, length, and time, during the lengthening or shortening of a retractor due to application of, or release from, loads can be simply represented by a mechanical model which includes a pure elastic system, a viscous-elastic system, and a pure viscous system controlled by a constant force, arranged in series.

I am indebted to Mr R. C. J. Howland for his kindness in solving a number of possibly relevant differential equations, and for his advice

on mathematical points. I also gladly acknowledge the help at various stages received from Dr L. E. Bayliss and Professors C. Lovatt Evans and A. V. Hill.

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ON THE MECHANISM OF SUGAR ELIMINATION IN PHLORRHIZIN GLYCOSURIA. A CONTRIBUTION TO THE FILTRATION-REABSORPTION THEORY ON KIDNEY FUNCTION.

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It was formerly assumed that the sugar in the blood was bound and non-diffusible (Lépine, Pavy). Its appearance in the urine then implied a specific secretion of glucose by the kidney cells. More recent investigations, however, have led to the conclusion that the blood sugar is freely diffusible. This was on general physiological considerations to be expected. The greatest theoretical difficulties were involved in the supposition that this most important food material did not freely permeate the capillary walls.

The fact that glucose normally does not appear in the urine could be attributed to a specific impermeability of the glomeruli to the glucose molecule, an impermeability that is known to exist in the case of the red blood corpuscles in the most widely different species of animals, and such a theory, founded on perfusion experiments with the frog's kidney, was a decennium ago presented by Hamburger and Brinkman(8). Hamburger perfused the frog's kidney from the aorta under a pressure of 60 cm. of water, and assumed that the fluid issuing from the ureters represented the pure glomerular filtrate. The results of his highly interesting experiments were that the percentage of glucose entering the urine was dependent on the interrelation between the amounts of cations in the Ringer solution employed, especially the ratio between the Ca and K ions; the correct proportion was assumed to tighten the glomeruli membranes, and excess of glucose in the perfusion fluid so to alter the membrane that the sugar leaked out. Against the interpretation that Hamburger offers for his experimental findings it may be objected that his assumption that the urine in his perfusion experiments was pure glomerular fluid is in no way justifiable; and it seems impossible to reconcile his theory with the modern view on kidney function. He

attributes to the glomerular membrane a specific impermeability for glucose, which can hardly be maintained in view of the general permeability of this membrane and the relatively high osmotic pressure of the blood sugar. This last Hamburger himself recognizes in his hypothesis that the fluid elimination in the glomeruli is due to a transudation in Cohnheim's sense, *i.e.* a combination of filtration, diffusion and osmotic action. Even the last assumption is not tenable according to the latest developments of our conception of the kidney function.

That Hamburger and Brinkman's explanation of their results is really not the correct one has been demonstrated by recent investigators in this field. With a very fine capillary tube Wearn(20) was able to puncture the capsular space and procure some fluid from a glomerulus in the frog's kidney. The fluid contained glucose while, at the same time, the urine in the bladder was sugar free. In the same year Clark(4) made some very valuable experiments on the frog's kidney. Under appropriate pressures he perfused the kidney from the renal artery and renal portal vein, and clearly demonstrated that the appearance of glucose from the glomerular filtrate in the elaborated urine was dependent on the concentration of sugar in contact with the tubule cells, which he was able to modify by changing the amounts of glucose added to the perfusion fluid sent through the renal portal vein.

The important results of Wearn's and Clark's work are that the glomerular fluid probably always contains glucose, and the non-appearance of this substance in the urine must be due to a quantitative reabsorption of sugar by the renal tubule cells.

Until evidence to the contrary has been obtained we are justified in assuming that the excretion of sugar by the mammalian kidney is, in the main, brought about in the same way as that found in experiments on frogs.

A peculiar and interesting form of glycosuria was observed by von Mering(15) in 1888. The glucoside phlorrhizin causes the urine of dogs into which it is injected to contain abundant amounts of sugar without the animals coincidently showing hyperglycæmia. Von Mering himself attributed the glycosuria to an increased permeability of the kidney for sugar. Some years later another explanation of the phenomenon was afforded by Levene(11), who assumed that the kidney during phlorrhizin poisoning actually produced glucose. Levene's theory has not been supported by modern work. The increase in the sugar concentration in the renal vein under phlorrhizin, the observation on which his assumption was based, Zuntz afterwards(23) attributed to experi-

mental errors, and has further not been verified by Nash jr. (16) in a recent series of experiments. Nash was unable to observe such an increase, and in some of his experiments he even found a decrease in the sugar content of the renal vein during the action of phlorrhizin. It may be computed from the amount of sugar excreted and the large blood flow through the kidney that this difference lay within the limits of experimental error. Strong evidence against renal sugar production is further brought forward by experiments on the isolated heart-lung-kidney preparation (de Boër and Verney(2)). Under these conditions the quantity of sugar found in the urine exactly corresponds to the extra amount removed from the blood after addition of phlorrhizin.

According to modern views of kidney function the probable explanation of phlorrhizin glycosuria is that the glucoside completely or partially prevents the tubules from reabsorbing glucose.

The correctness of this idea may be experimentally decided. Rehberg(18) recently made it highly probable that creatinine is typically what in renal physiology is termed a "no-threshold" body, *i.e.* a substance that appears in the urine in the amount in which it is present in the glomerular filtrate. This conception was already formulated in an important paper by Mayrs(13), and the same author has further shown what has been since confirmed by Poulsson(17) that inorganic sulphate in the kidney behaves in much the same way. In regard to threshold bodies, *i.e.* substances that disappear from the urine during its passage through the tubules, Rehberg has introduced a distinction which admirably supplements the filtration-reabsorption theory advanced by Ludwig and Cushny. When a substance passes back to the blood through the tubule cells this may be due either to active reabsorption by the tubule cells or merely to passive diffusion resulting from differences in concentration of the substance on the two sides of the thin walls of the tubules. In the case of the threshold body chlorine both these factors are conspicuous, whereas the fact that urea is concentrated by the kidney to a less degree than inorganic sulphate or creatinine, must be attributed to mere diffusion.

If the sugar during phlorrhizin poisoning is filtered through the glomeruli in the same concentration as obtains in the plasma, and neither is reabsorbed nor diffuses back during the passage through the tubules, we should find the same concentration ratios for glucose and for creatinine. If phlorrhizin eliminates only active reabsorption of sugar while the glucose still diffuses back in some degree, it consequently will be concentrated to a less extent according to the degree of diuresis,

but the concentration index of sugar will vary in accordance with that of creatinine, as demonstrated by Rehberg in the case of urea.

Two investigations bearing upon this question are reported in the literature. Mayrs(14) observed that after intravenous infusion of sulphate into rabbits treated under urethane with phlorrhizin, 0·2 g. per kg., the sulphate ion was concentrated by the kidneys 0·98–1·91 times more than the sugar, and offers the explanation that phlorrhizin only partially prevents the reabsorption of glucose by the renal tubule cells. White(23), who advocates the secretion theory, in a dog under morphia and ether and the same dose of phlorrhizin, observed a close connection between the figures for plasma sugar and the absolute amounts of glucose excreted in the urine. His findings clearly point to filtration. The result of another experiment indicates that sugar is concentrated by the kidney somewhat more (1·17–1·80) but closely parallel to the inorganic sulphate. Probably his method for the estimation of sulphate in plasma yields figures that are a little too high.

Experimental.

The aim of my own researches was to study the quantitative relation between plasma and urinary sugar in alimentary glycosuria produced in normal individuals, and in phlorrhizin diabetes. The first part of the investigation had to be abandoned. Like Folin and Berglund(6) I found it impossible to produce glycosuria even after excessive amounts of glucose by mouth. Two experiments on myself were done. In the first on an empty stomach 100 g. of glucose dissolved in 500 c.c. of tap water was drunk at 8.45 in the morning, the same dose was repeated at 10.35 and at 12.10. No glycosuria occurred. In the next experiment 100 c.c. of a 25 p.c. glucose solution was taken every fifteen minutes for 3 hours beginning at 9.15. In the urine samples from the first 2 hours doubtful traces of sugar seemed to be present (Benedict's reaction), but it did not seem worth while to continue the experiments as the glycosuria later on disappeared and there was not sufficient diuresis to get frequent samples of urine.

The second part of my investigation concerning phlorrhizin glycosuria is reported here. Two female dogs were used. On the day preceding the experiment food was withheld from 15.00 and only water ad libitum allowed. I used phlorrhizin (Merck) in doses from 3·8 to 5 g., *i.e.* somewhat more than 0·2 g. per kg. The glucoside was given in about 20–25 c.c. of water to which was added just enough 10 p.c. sodium carbonate solution to give a clear solution in the cold. Injections were administered subcutaneously 2 hours before the collection of urine and blood began.

Creatinine (B.D.H.) 4 g. was given in about 300 c.c. of water by stomach tube, and in order to get urine of widely different concentration water was supplied in the same way during the experiments. The urine was obtained by catheterization and careful expression of the bladder every hour, blood was obtained from the veins on the hind and fore legs exactly midway between the two catheterizations. The experiments lasted for 5 hours.

Methods.

As anti-coagulant heparine was employed; this preparation has for the last few years been almost exclusively used in this laboratory. It was ascertained that heparine has no influence on the reduction values in blood (three samples of blood from myself, with oxalate, fluoride and heparine respectively, gave 89 mg. p.c. glucose). To avoid a possible shifting of blood constituents between the red corpuscles and plasma, a rapid centrifuge (5000–6000 revolutions per minute) was used and only 3 to 5 minutes elapsed between the venous puncture and pipetting the plasma for analysis. In this creatinine was determined according to Rehberg's modification(18) of Folin's original method, glucose by the method of Hagedorn and Norman Jensen(7). To get the real content of sugar in plasma during phlorrhizin poisoning in some cases, the residual reduction was actually determined after fermentation of the glucose according to Hemmingsen(9). Hagedorn(7) has found that creatinine has a reduction value for ferricyanide of about 50 p.c. of glucose, corrections for plasma creatinine were accordingly introduced.

The determination of creatinine was performed in the appropriate dilutions of urine in 100 c.c. flasks and 1·5 mg. standards (Folin); the presence of glucose (I tried 5 p.c.) does not modify the colour. Estimation of sugar in urine was made according to Benedict(1).

A preliminary experiment showed the efficiency of different methods

Table I.

Urine treated with	Creatinine removed (p.c.)	Glucose found (p.c.)	Colour of filtrate
(1) Carbo medicinalis (Merck) after addition of hydrochloric acid to 10 p.c.	31·7	1·52	Water clear
(2) Kaolin	5·7	1·72	Somewhat more yellow than (5)
(3) Fuller's earth	23·5	1·75	„
(4) Mercuric nitrate according to Patein and Dufau. Hg in filtrate removed with Zn and HCl	60·0	1·57	Water clear
(5) Lead acetate	2·4	1·65	Faintly yellow
(6) Siliceous earth	4·5	(1·91)	Only slightly decolorized

for the removal of colouring matter and creatinine before the sugar titrations in urine. Glucose (Merck's *pro infusione*) 1·56 p.c. and some creatinine were added to a very concentrated specimen of human urine. Determination of creatinine—0·822 p.c.

The table indicates, as especially emphasized by Ivar Bang, that urochrome interferes to a certain degree with the sugar titration. In my own investigations I adopted the Pateln and Dufau procedure, and removed the mercury in the filtrates by shaking them vigorously for 10 to 15 minutes with zinc dust after the addition of five drops of concentrated hydrochloric acid.

Extracts of my experiments are given below.

Exp. 1. Stella. Weight 17·2 kg. 8.15, 4 g. phlorrhizin subcutaneously: 9.00, 4 g. creatinine in 300 c.c. of tap water: 10.00, bladder emptied and urine samples taken every hour till 15.00: venous puncture 10.30 and every hour till 14.30: 10.58, 300 c.c. and 13.02, 150 c.c. of tap water by stomach tube. From 9 till 10 o'clock the dog had some diarrhœa and was apparently unwell.

Table II gives the results of sugar and creatinine determinations. Residual reduction assumed to be 8 mg. p.c., this amount and 50 p.c. of the plasma creatinine in mg. is subtracted from the original reduction values observed.

TABLE II.

	Urine vol. in c.c.	Sugar			Creatinine			
		In plasma mg. p.c.	In urine mg. p.c.	Concentration	In plasma mg. p.c.	In urine mg. p.c.	Concentration	$\frac{\text{C. sugar}}{\text{C. creat.}}$ p.c.
1.	23	112	8080	67·4	14·64	1249	85·4	79
2.	62	94	2870	30·5	16·05	571	35·5	86
3.	269	89	833	9·34	15·72	162	10·3	91
4.	192	94	1083	11·52	14·64	192	13·15	88
5.	83	85	2250	26·5	13·00	385	29·6	90

If on the basis of the creatinine analysis, according to Rehberg's formulæ

$$F \times \frac{\text{C. creat. plasma p.c.}}{100} = U \times \frac{\text{C. creat. urine p.c.}}{100}, \text{ and } R = F \div U,$$

we compute the amounts of fluid and glucose filtered and absorbed per minute, and the concentration of sugar in the reabsorbed fluid, we get the following data:

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	32·7	32·3	36·6	5·5	17·0
2.	36·7	35·7	34·5	5·8	16·2
3.	46·2	41·7	41·2	3·9	9·4
4.	42·1	38·9	39·6	5·5	14·1
5.	42·0	40·6	35·8	4·6	11·3

Exp. 2. Lisa. Weight 18 kg. 8.05, 3·9 g. phlorrhizin: 9.00, 4 g. creatinine in 300 c.c. water: 11.05, 500 c.c. of tap water by stomach tube: 10.00, bladder emptied, urine and blood samples as in Exp. 1. The dog seems to be quite well. No albuminuria.

Residual reduction in blood sample 1 = 17 mg. p.c., *i.e.* 17 mg. p.c. ÷ 0·5 × 16 mg. p.c. = 9 mg. p.c. without creatinine.

TABLE III.

	Urine vol. in c.c.	Sugar: In plasma mg. p.c.	Sugar: In urine mg. p.c.	Sugar: Concentration	Creatinine: In plasma mg. p.c.	Creatinine: In urine mg. p.c.	Creatinine: Concentration	$\frac{\text{C. sugar}}{\text{C. creat.}}$ p.c.
1.	160	104	1960	18·7	16·35	320	19·6	95·5
2.	166	108	1345	12·5	14·06	210	15·0	83·4
3.	272	99	840	8·5	12·33	127	10·3	82·5
4.	93	101	2270	23·0	11·05	327	29·6	77·8
5.	49	85	4025	47·3	9·03	544	60·2	76·4

Calculation according to Rehberg shows:

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	52·2	49·5	54·6	2·3	4·6
2.	41·5	38·7	44·8	7·6	20·2
3.	46·7	42·2	46·2	8·1	19·2
4.	45·9	44·3	46·4	11·2	25·3
5.	49·4	48·6	42·0	9·1	18·7

As will be seen from Exps. 1 and 2 a close parallelism exists between the concentration ratios of glucose and creatinine, the concentration of sugar by the kidney however is somewhat less efficient. The possible explanation may be that the phlorrhizination in these experiments was incomplete or perhaps that the blood samples from the leg vein do not give the correct expression regarding the glucose content in the renal artery. Probably the glucose-drainage through the kidneys under phlorrhizin leads to an emission of sugar not only from the liver but in part from the tissues. My tables show that the concentration of glucose in plasma is only slightly if at all reduced after injection of the glucoside.

Exp. 3. Lisa. Weight 18 kg. No food restriction during the day and night before the experiment. 8.15, 5 g. phlorrhizin: 8.35, 4 g. creatinine in 200 c.c. of water. The experiment was performed as 1 and 2, with the exception that the blood samples were taken from the ear vein after the shaved ear had been vigorously rubbed with toluene in order to paralyse the vessels, and to prevent coagulation a little vaseline was applied before the incision in the marginal vein. The blood was bright arterial in colour. Duplicate analyses of the residual reduction figures were made after Hemmingsen; the samples were incubated at 34° C. for 54 hours.

Values found: (1) 23 mg. p.c., (2) 15 mg. p.c., (3) 10 mg. p.c., (4) 12 mg. p.c., (5) 8 mg. p.c. During the drawing of the blood samples 3 and 4 the dog was slightly excited. At 12.02 the animal received 500 c.c. of water by stomach tube.

The result of this experiment is summarized in Table IV.

TABLE IV.

	Urine vol. in c.c.	Sugar: In plasma mg. p.c.	Sugar: In urine mg. p.c.	Sugar: Concentration	Creatinine: In plasma mg. p.c.	Creatinine: In urine mg. p.c.	Creatinine: Concentration	$\frac{\text{C. sugar}}{\text{C. creat.}}$ p.c.
1.	147	86	2035	23·5	18·25	393	21·5	110
2.	57	94	4750	50·6	15·38	856	55·6	91·2
3.	196	93	1450	15·6	11·72	207·5	17·7	88·1
4.	210	85	1335	15·7	9·68	152·7	15·8	98·8
5.	58	91	4490	49·4	7·60	453	59·6	83·0

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	52·8	50·3	45·4	[−4·4]	[−8·7]
2.	52·9	51·9	49·7	4·6	8·9
3.	57·9	54·6	53·9	6·7	12·3
4.	55·3	51·8	47·0	0·3	0·58
5.	57·7	56·7	52·5	7·7	13·3

In a previous investigation the author has gathered some evidence in favour of the opinion that pituitrin acts on the cells in the renal tubules. To determine if the phlorrhizin in some way interferes with the anti-diuretic action of pituitrin the following experiment was performed.

Exp. 4. Stella. Weight 17·1 kg. 8.00, 3·9 g. phlorrhizin. At 9.00, bladder emptied and urine sampling started every hour to 14.00. No blood samples.

9.00, 200 c.c. water by stomach tube
11.00, 300 c.c. „
12.00, 300 c.c. „
12.05, 5 units pituitrin injected subcutaneously.

TABLE V.

	Urine vol. c.c. per hour	Sugar in urine g. per hour
1.	41	0·71 (action of phlorrhizin evidently not yet complete)
2.	135	1·803
3.	200	2·150
4.	120	3·435
5.	70	2·740

The usual anti-diuretic action of pituitrin is obviously present. The increase in the sugar excretion is certainly due to the rise of blood sugar following injections of pituitrin. As demonstrated by Erlandsen(5) the phlorrhizin glycosuria is likewise augmented when hyperglycæmia is induced by bleedings or injections of adrenaline.

Discussion.

The result of my investigations is that a close agreement has been shown to exist in the behaviour of glucose in phlorrhizin poisoning and that of the typical no-threshold substance creatinine. This observation has a bearing upon two questions. In the first place, my findings lend some support to the filtration-reabsorption theory of kidney function. It is now known that three such fundamentally different substances as creatinine, inorganic sulphate and glucose in certain conditions behave in the kidney similarly. This seems to me to give a final proof of the filtration-reabsorption hypothesis now extensively adopted by leading physiologists.

The second point of some interest is that phlorrhizin apparently completely paralyses the reabsorption of glucose in the renal tubules. The data from my Exp. 3 indicate a very close correspondence between the concentration ratios of sugar and creatinine; in the first sample the glucose is even concentrated 10 p.c. more than creatinine.

The findings in Exp. 3 are put together in Fig. 1.

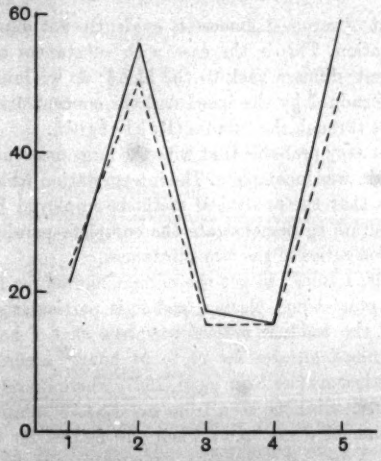

Fig. 1. The ordinate represents the concentration indices ——— of creatinine, and - - - - - - of sugar. The abscissa indicates time.

When we try to interpret the finding that glucose is in most cases

concentrated somewhat less than creatinine the following explanations offer themselves.

The sugar might in some degree during the concentration process diffuse back into the blood through the tubule walls. After what is already known about the rate of diffusion of glucose (von Brasol(3), Leathes(10)), this is not very probable. To throw some light on the question, Fig. 2 is constructed.

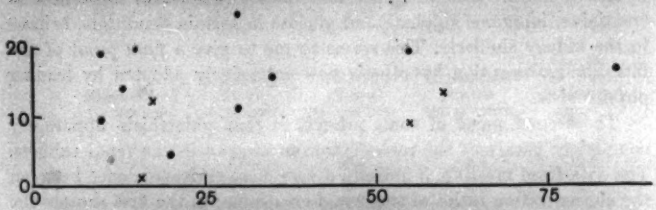

Fig. 2. Ordinate: mg. p.c. glucose in reabsorbed fluid. Abscissa: concentration of urine according to the creatinine determinations.
● Exps. 1 and 2. × Exp. 3.

The amount of retained glucose is evidently not dependent on the urine concentration. This is the case with substances as urea which, to a great extent, diffuses back to the blood; as we must expect, this diffusion is determined by the speed and the concentration with which the urine passes through the tubules (Rehberg(18)).

It is also not very probable that with the large doses used, the action of the phlorrhizin was incomplete. The interpretation which seems most likely to me is that the analytical methods employed have not been sufficiently accurate to demonstrate the complete parallelism between the concentration ratios of the two substances.

It is difficult, I think, to get the correct figures for the amount of glucose in the renal blood plasma, and it is particularly in regard to the figures for the residual reduction values that I have doubts. I incubated the blood filtrates for 24 to 54 hours; according to recent American investigators (see Somogyi, 1927, where literature is quoted) this is too long. As will be seen from my Exp. 3 sample 5 showed a residual reduction of 8 mg. p.c.; when the figures for creatinine are subtracted only 4 mg. p.c. remains, a value evidently too low. Finally, too, it is possible that a few mg. p.c. of the sugar in the plasma is non-diffusible. As already pointed out, however, I believe that the observed differences in concentration ratios between creatinine and glucose are not

real, and the expressions "mg. reabsorbed glucose" and "mg. p.c. glucose in reabsorbed fluid" in this connection consequently are misleading.

It has been a matter of some dispute (Loewi(12), Weber(21)) whether a superimposed diuresis increases the sugar elimination in phlorrhizin diabetes. This, of course, depends on whether the extra diuresis is accompanied by changes in the rate of filtration through the glomeruli or not. When the filtration is augmented, *e.g.* by raising the pressure in the renal artery in the heart-lung-kidney preparation (de Boër and Verney) the amount of sugar in the urine is also increased; if the rate of filtration remains very constant, as is the rule normally, the sugar elimination like the urinary content of inorganic sulphate and creatinine is independent of variations in the volume of water excreted. Fig. 3 (Exp. 3) illustrates this point.

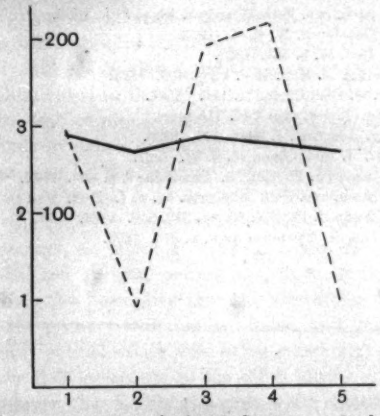

Fig. 3. Ordinate: ------ c.c. of urine per hour, —— g. glucose excreted per hour. Abscissa: time in hours.

Summary and conclusions.

During phlorrhizin poisoning in unanæsthetized dogs, the kidneys concentrate the glucose approximately in the same degree and in close parallelism with creatinine. This observation supports the Ludwig-Cushny view on kidney function, and furnishes evidence that phlorrhizin completely paralyses the renal tubules as to their power of reabsorbing sugar.

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LATENCY OF AFTER-IMAGES AND INTERACTION BETWEEN THE TWO RETINO-CEREBRAL APPARATUSES IN MAN.

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Introduction.

Since 1882, when Parinaud(12) called attention to the phenomenon, several investigators have studied the after-images which are seen to follow suitable stimulation of the retina of one eye if, at the end of the fixation period, that eye be closed and the gaze of the other be directed to an evenly illuminated surface. Parinaud concluded from his experiments that the seat of after-images is cerebral and not retinal; and Bocci(2, 11) finds evidence in similar observations for supposing that the after-image seen by the "resting" eye (*i.e.* the eye which did not receive the primary image) is of cerebral origin, and differs in various respects from the ordinary negative after-image seen by the "active" eye. The arguments, on which Parinaud based his conclusion, have been justly criticized by later writers (4, 5, 10, 8) on the ground of his failure to exclude the possibility that the after-image belonged to the eye on which the primary stimulus had fallen, and that such an after-image of possibly retinal origin was either combining or entering into rivalry with the field presented to the other (open) eye. It should be mentioned, however, that Ebbinghaus(5), while regarding Parinaud's observations as valueless from this point of view, described the appearance, under comparable circumstances, of a positive after-image possessing properties incompatible with the obvious interpretation of a combination or rivalry between the negative after-image of the active eye and the bright screen held before the resting eye.

Although, therefore, observations of this type can probably throw little light on the question of the "seat" of after-images, and the experiments described below were not designed to elucidate that problem, it seemed to us that the method might profitably be employed for another

purpose—a hope which was encouraged by some of the findings incidentally reported by the earlier workers.

By a study of the latent periods preceding the development of the negative after-images of white circular discs, Creed and Granit(3) were able to demonstrate functional differences in the receptor apparatus of the foveal and para-foveal areas of the retina, and to obtain evidence of interaction between the mechanisms concerned in the reception of visual stimuli at varying angular distances from the fixation point. The present enquiry extends this work to the functional relations between the retino-cerebral apparatuses of the two eyes in man, thereby supplying data on the mechanism of binocular vision.

We decided to investigate how the time-relations of the negative after-image of a simple white object, seen uniocularly, might be modified by projecting the after-image on to a screen presented to the other eye. The results of this procedure are reported in Part II below. It was first necessary, however, to make some preliminary observations, described in Part I, on the influence of certain other factors on the time-relations, and particularly that of the luminosity of the projection field. For it is obvious that, if the primary image falls on the right retina and the after-image is projected on to a screen seen only by the left eye (the right eye being now covered), the conditions are not directly comparable with those existing when the right eye is exposed both to the object and to the white screen. Comparison must also be made with the effects of looking at the object with the right eye and then, at the end of the fixation period, covering this eye without simultaneously exposing the left eye to the screen (we call these "black projection" experiments). In Part III of our results, some observations made under slightly more complicated conditions are recorded.

Method.

The technique adopted was much the same as that of Creed and Granit(3), and need not again be described in detail. Observations were made in a dark room illuminated by an opal-glass electric lamp of about 80 c.p. Before all experiments, observers waited for 30 minutes so that their eyes might become adapted to the illumination. The test objects—white circular discs provided with a central fixation point—were placed (except when otherwise stated) 4 metres from the lamp, and were surrounded by the uniformly black wall of the room. After closing his eyes for 30 seconds, the observer fixated the object from a distance of 1·50 metres for 30 seconds. The disc was then either covered by a large

white screen (of the same brightness as the disc) provided with a fixation mark, or, for "black projection" experiments, the light was switched off. The interval which elapsed before the appearance of the negative after-image was measured with a stop-watch.

When the disc had been viewed uniocularly and the other eye only was required to see the screen, various devices were employed. For small discs the observer generally looked through a box, 15 cm. square, blackened inside, and with two holes cut for the eyes at one end and corresponding holes at the opposite end. Running down the middle of the box, in a line joining the observer's nose and the fixation point, was a partition, so that the arrangement was virtually one of two black tubes. A black cloth over the observer's head reached forwards on to the top of the box, and shut out from view everything except what was visible through the box. The holes at the far end could be covered singly or together with small black curtains, or alternatively a shutter could be pushed across from one to the other. These adjustments were under the control of the observer, who made them as required the moment the dropping of the screen by the experimenter told him that the fixation period was at an end. The field of vision through these boxes measured about 36 cm. by 26 cm. at 1·50 metres. The area of projection field surrounding the after-images of some of the larger discs employed seemed, therefore, insufficient, and so we covered and uncovered the eyes with our hands and a black cloth in several experiments.

These methods were also used in some of the observations in which the after-image was projected on a dark field and, indeed, in most of our later work whenever it was desired to compare such black projection images with images projected by one eye following stimulation of the other. We thought it advisable to satisfy ourselves that no great error was introduced by using two distinct methods for black projection experiments. We therefore each made six determinations of the latent period preceding the after-image of a 26 cm. disc. In alternate observations the black projection field was obtained (*a*) by the experimenter switching off the light, and (*b*) by the observer covering his eyes the moment the experimenter began to drop the screen. For each of us, the means of the results showed that the image appeared 0·2 second earlier with (*a*) than with (*b*) (Observer C. 5·1 and 5·3; Observer H. 2·8 and 3·0). The difference between the two is therefore negligible for our purposes.

To eliminate errors due to fatigue, the duration of each experiment was limited to 1 hour, during which time each observer made about

twelve observations. Although it is sometimes stated that after-images are best seen early in the day, we often found that our measurements were more consistent and more reliable in the evening than in the morning.

Results.

Part I. *Preliminary observations on the effect of certain factors on the latency.*

A. *Influence of brightness of projection field.*

The only previous systematic investigation of this factor appears to be that of Juhász(7), who found that the darker the surface on to which the complementary after-image of a bright coloured object, binocularly fixated, is projected, the longer is the latency and the longer is the duration of the after-image. Observations recorded incidentally by Bocci(2, 11) also indicate that negative after-images develop more slowly when both eyes are closed after uniocular fixation of an object than when the gaze of the "active" eye is transferred to a white surface.

Our experience has uniformly supported these findings in that black projection images have shown a longer latency than those projected on the white screen. The following are a few examples. For convenience, *R*, *L*, and *Bin.* are used to signify observations in which the right eye only, the left eye only, and both eyes together were respectively kept uncovered throughout the experiment. The addition of *Black proj.* means that the after-image was projected, as described above, into a dark void. The latencies are given in seconds, and the observations read from left to right in temporal sequence.

| Observer C. 21. ix. 1928. 26 cm. disc | | |

It may be objected that with our technique (although not with Juhász) the black projection experiments differ from all others in the absence of a fixation point during the after-image period, and that the widely held view that after-images are readily inhibited by ocular movements provides a simple explanation for the delay noted above. The following observations were designed to test this point and show that the latency is not so affected. In one side of a closed box, a circular hole, less than 2 mm. in diameter, was made. The centre of a disc was placed over this hole, which constituted a suitable fixation point capable of being illuminated from behind by a small electric bulb within the box. The disc was observed as usual for 30 seconds and then the 80 c.p. lamp illuminating its face was switched off. In alternate observations the fixation point was lighted and was black. In the former case, fixation was easily maintained on the bright spot in an otherwise completely black field of view; in the latter case the conditions were identical with those in our other black projection experiments.

Observer C.

25. iii. 1929. 12 cm. disc, 2·10 m. from lamp

	Bin. Black proj.				L Black proj.
Fixation point illuminated	3	3·2		2·8	11
Fixation point black	3	3·4	2·6		11

The only detectable difference resulting from the presence of a fixation point was that the first phase of the after-image lasted longer. Thus in the above experiment the binocular after-image had a first phase of 25 to 27 seconds when the fixation point was illuminated, as compared with 6 to 8 seconds when the field was completely black. The corresponding figures for the *L* image were 9 and 6.

Nor can it be said that black projection images are reported later than white projection images merely because they are more difficult to see against a background of darkness, and hence do not so rapidly reach the threshold of consciousness. The evidence to the contrary is overwhelming. It is only necessary to mention that, if this were so, the first phase of the black projection image (which resembles the ordinary negative image in being periodic) ought to be visible only during the middle of the corresponding phase of the white projection image. This has not been our experience. The moment of disappearance of the black projection image is commonly considerably later than that of the corresponding image on the white screen, and (in Observers C., H., and T.) we have not infrequently found, especially in uniocular experiments, that the

black projection image does not even begin until a time when the first phase of a white projection image would have faded.

Lastly, a few remarks must be made about the phenomenology of these images. They differed from images projected on to the white screen mainly by being surrounded by a much more distinct and brighter halo, and by the fact that (except when they were coloured) they were more intensely black. The black projection after-images of large discs (26 cm. diameter) surprised us by appearing bright blue in their first phase, with the usual brilliant halo separating them from the uniform blackness of the rest of the visual field. But, in spite of the fact that they were more luminous than the general background, there can be no doubt that they were of the same essential nature as the intensely black negative images of the smaller discs. They developed in the same steady way surrounded by a strong halo; the blue was found to deepen and become blacker as time went on; phases subsequent to the first were often pure black; with smaller discs (*e.g.* 12 cm.) the black first phase of the black projection image was sometimes tinged slightly with bluish purple; Observer C. noted that the *Bin. Black proj.* image was bright blue, the *R Black proj.* image blue-black, and the *L Black proj.* image black; there is, in short, a continuous series between the ordinary negative images and these coloured ones.

Other evidence that our method of experiment was unfavourable to the production of persistent positive images was provided by some observations with a red disc, 11 cm. in diameter. Whether projected on to the white screen or into darkness, its after-image was green, surrounded in the latter case by a reddish halo. But, as Creed and Granit had also described, positive images sometimes appeared before the negative, especially with large discs. Observer H., in particular, saw them very frequently in black projection experiments (more often in binocular than in uniocular observations), and they gradually gave place to the negatives. Both of us found that the positive had an ill-defined edge, and seemed to include the halo as well as the disc, so that when the negative later developed, it was as a black circle within the borders of the bright positive. McDougall(9) has recorded somewhat similar appearances.

B. *Comparison of uniocular and binocular images.*

Images projected on to a white screen. As the following figures show, it makes little or no difference to the latency of the after-image whether images of the disc and screen are formed on both retinæ or on one only. Nor in our experience are the duration of the first phase and the occur-

rence of later phases perceptibly influenced thereby. We have, however, made no systematic study of the latter points, and it may be that the uniocular after-images are slightly less intense than the binocular.

Observer C.

	6. ix. 1928. 2 cm. disc (fixation period 15 sec.)			7. ix. 1928. 8 cm. disc (fixation period 15 sec.)	
Bin.	6·4	6	8	3·4	3·4
R	6			3	
L	6				

	5. ix. 1928. 10 cm. disc (fixation period 15 sec.)		7. ix. 1928. 12 cm. disc (fixation period 15 sec.)	
Bin.	3	3	2·8	2·8
R	3			
L	3·4		3	

	3. ix. 1928. 20 cm. disc (fixation period 15 sec.)			25. ix. 1928. 26 cm. disc		
Bin.	3·2	3	1·8			
R	3·8			2		2
L	3·2			3	2	

Observer H.

	6 ix. 1928. 4 cm. disc (fixation period 15 sec.)				11. ix. 1928. 6 cm. disc (fixation period 15 sec.)			
Bin.	3·2	4·2	3·2	3·2	3			3·8
R	3				2·8	3		
L	3·8				2·4	2·6		

	5. ix. 1928. 10 cm. disc (fixation period 15 sec.)			6. ix. 1928. 14 cm. disc (fixation period 15 sec.)		
Bin.	2·4	2·8	2·8	2·2	2	2
R	2·8			2		
L	3·2			2		

	6. ix. 1928. 26 cm. disc (fixation period 15 sec.)			25. ix. 1928. 26 cm. disc		
Bin.	2·2	2	1·8	2		
R	1·8			2		2
L	2			2	2	

Black projection images. On the other hand, if only one eye has been exposed to the disc, the after-image subsequently seen in complete darkness differs markedly from that following binocular fixation. To begin with, the uniocular after-image is more difficult to obtain, and with fixation periods shorter than 30 seconds, frequently fails to appear at all. It is always less distinct, less black, with a narrower and less intense halo, and of shorter total duration than the corresponding binocular after-image. Whereas the latter often shows at least four distinct phases before it finally vanishes, it is exceptional, with our technique, to see more than one phase of the former. Sometimes, too, the uniocular image is incomplete, only a small segment of sharp edge

being visible with the corresponding part of the halo. In Observers C. and H., images following fixation with the *L* eye were generally fainter, and showed a greater tendency to incompleteness than those in which the primary image had fallen on the *R* retina. It was mentioned above that in Observer C. *R Black proj.* images of large discs were sometimes coloured, while *L Black proj.* images were black.

But, although the uniocular after-images were sometimes faint, they were not as a rule difficult to time. On cutting off the illumination, the field remains dark for a relatively long period, and then the image surrounded by its bright halo appears quite suddenly and unmistakably. Under such circumstances timing can hardly fail to be accurate, and Observer C. has confidence in his figures reproduced below. Except where the occurrence of a positive image is noted, he saw nothing before the arrival of the negative after-image. It will be seen that the latent periods preceding the uniocular images are much longer than those preceding the binocular, and that usually (though not quite invariably) the *R* images occur earlier than the *L*. The end of the first phase of the binocular after-image was also consistently much earlier than that of the uniocular.

Observer C.

	13. ix. 1928. 10 cm. disc						21. ix. 1928. 12 cm. disc					
Bin. Black proj.	4·4		4			4·2	3·2		4·4			3·8
R Black proj.		10·2			13·2			13·2		9		11·4
L Black proj.			12·2	13·6		17·6				12	15	

	22. ix. 1928. 26 cm. disc						26. ix. 1928. White screen (64 cm. × 51 cm.)					
Bin. Black proj.	2·6		6·2*			4·2*	3·2		6·4			
R Black proj.		11			5·2			12·2			11·2	
L Black proj.			13	12					13·8			13

* Preceded by a positive.

Observer T., on whom a few experiments of this type were performed, gave similar results, except that the latency of *R* uniocular images was longer than that of *L*. This is a point of some interest to which we shall return later.

Observer T.

	17. xii. 1928. 26 cm. disc				25. iii. 1929. 22 cm. disc					
Bin. Black proj.			3·2			2·8				
R Black proj.		6·2			5·2			(3)		4·2
L Black proj.	4·2					4·2			4·2	

In Observer H., however, a series of observations, extending over ten separate experiments, failed to demonstrate that the interval preceding the appearance of the dark image was consistently any longer in uniocular than in binocular tests. On only one of these

occasions were the shorter latencies of the binocular images unmistakable; in the remainder there appeared to be little difference between the two. Possibly this may be connected with the fact mentioned earlier that this observer was prone to see positive images for some time before the negatives appeared, and indeed saw them almost constantly in binocular black projection experiments. The positive may, in some way, have postponed the arrival of the negative (as Observer C.'s figures given above suggest), with the result that the binocular images were commonly delayed more than the uniocular. It is not the purpose of the present paper to discuss the bearings of our experiments on theories of after-image production, but it is germane to note that, according to some of these theories, for example McDougall's(9), there is no essential difference between the mode of causation of positive and negative images. If this be true, we obviously ought, in our experiments, to have timed the first appearance of any after-image, quite irrespective of its type, instead of confining our attention to the dark image surrounded by a bright halo. This has been done in one experiment with the following result:

Observer H.

3. ii. 1930.

		10 cm. disc.		26 cm. disc
Bin. Black proj.	4·4 neg.	2·8 pos. (neg. *c.* 2 sec. later)	2·8 pos. (neg. *c.* 2 sec. later)	1·7 pos. (neg. *c.* 1·5 sec. later)
R Black proj.	8·8 neg.			3 pos. (neg. *c.* 1 sec. later)
L Black proj.		6·9 neg.		2·8 pos. (neg. *c.* 1 sec. later)

In this case, the same qualitative result, in agreement with the experience of Observers C. and T., would have been obtained by measuring only the negatives. In most of H.'s observations, *L Black proj.* have shown a longer latency than *R Black proj.*, but he has nearly always found these uniocular images difficult to time and rather indefinite. Those recorded above, however, were quite definite and distinct.

Part II. *Primary image received by one eye and after-image projected on white screen seen with the other eye.*

When Parinaud(12) closed his *L* eye, and fixated for a few seconds with his *R* a red circle lying on a sheet of white paper, he found that the whiteness of the field became less intense and the colour of the disc less distinct. If he then removed the circle and continued to gaze at the white surface with his *R* eye, he saw a green after-image, brighter than the background, "sinon instantanément, du moins d'une manière très immédiate." If, on the other hand, at the moment when he removed the circle he closed his *R* eye and opened his *L*, and then maintained his gaze on the paper as before, "l'image du cercle n'apparaît pas immédiatement. Le blanc du papier s'obscurcit tout d'abord, et c'est seulement alors que l'image se dessine colorée en vert et plus claire que le fond." In other words, the latency of the *R* after-image was shorter

than that of what we shall call the $R \rightarrow L$. Bocci(2, 11) obtained similar results on projecting the after-image of a black square surrounded by a white background on to a white or grey field.

These findings of theirs have been confirmed in nearly all our experiments, the technique of which differs in one important respect from theirs, viz. that our white discs were set on a dark background[1]. The following are a few illustrative examples.

Observer C.

	15. ix. 1928. 10 cm. disc							25. ix. 1928. 26 cm. disc						
R→L		4·4			5·4		5			3·4				3·6
L→R			8			7·8		7·4				4·8		3·4
R	3·6			3·2			2·8			2			2	
L		3·8		3·8				4·4		3		2		

Observer H.

	17 ix. 1928. 10 cm. disc							25. ix. 1928. 26 cm. disc						
R→L		6·2			3·8	4	4			2·8				2·6
L→R			9	9·8		13			nil			3·2		4·8
R										2			2	
L											2		2	
Bin.*	3·8		4·8				4·8	4·6		2				

* It was shown in Part I that the values of *Bin.*, *R*, and *L* may be taken as identical.

	Observer L. 2. iii. 1929. 26 cm. disc						Observer T. 17. xii. 1928. 26 cm. disc					
R→L	3			3		3·2	3·8		4·4		3·4	
L→R		1·8			2	2		3·8		5·2		2·6
R	<1						2					
L			<1									2

In view of the phenomenology of $R \rightarrow L$ and $L \rightarrow R$ images, however, we do not regard the above results as of much interest or importance. In our earliest experiments we noted that $R \rightarrow L$ and $L \rightarrow R$ images differed from *R* and *L* images in that they were blacker, and had a much more obvious broad white halo. We noted, too, that the whole of the screen, with the exception of a small area surrounding the fixation mark and the portion occupied by the halo, suddenly became darker when the after-image developed, to brighten again to its normal white appearance when the after-image faded. When, later, we experimented with *Black proj.* images, we at once recognized (as also did Observer T.) their similarity to $R \rightarrow L$ images. It was clear that what we were seeing in the latter case was a *R Black proj.* image entering into rivalry with the white screen seen by the *L* eye, and temporarily displacing it in a marked degree from consciousness. Only thus can the darkening of the screen be

[1] Alterations in the background are known to influence the latency of after-images(7), and this may account for some apparent discrepancies between some of Bocci's rather complicated experiments and our observations comparing *R Black proj.* with $R \rightarrow L$ images.

accounted for. And on *a priori* grounds as well, it would seem more logical to compare a $R \rightarrow L$ image with a *R Black proj.* image than with a *R* (or *L*) white projection image.

Of fifteen experiments in which we have made comparative measurements of the after-images under these two sets of conditions, all but one (Observer H., one of three experiments with a 26 cm. disc) have shown the latencies of $R \rightarrow L$ images to be shorter than those of *R Black proj.*, and the latencies of $L \rightarrow R$ to be shorter than those of *L Black proj.* If the ends of the first phases are also timed in the two cases, it is found that the "offs" behave in the same way as the "ons." We are thus led to the conclusion that when a uniocular black projection after-image is in rivalry with a white field presented to the other eye, it appears earlier than when, in the absence of any stimulus to the other retina, it might be expected to develop with less hindrance. The following are some of the values actually obtained:

Observer C.

	14. ix. 1928. 10 cm. disc				27. ix. 1928. 12 cm. disc					
R→L		7·4		6			4·8		4·8	5·6
R Black proj.	9·8		8·2			10·4		12·4		17·8
L→R	10·6		9·6				8·4			
L Black proj.		15·6		21·8	12·2		13·8			

	27. ix. 1928. 26 cm. disc								
L→R		6·4		5·2	6		5·8		(Average 5·85)
L Black proj.	13·6		18·6		18	11·8		11	(Average 14·6)

Observer H.

	8. ix. 1928. 10 cm. disc		27. ix. 1928. 12 cm. disc				22. ix. 1928. 26 cm. disc			
R→L	3·8			2·8		3	2·8		3	
R Black proj.		4·8	3·4		4	3·4		3·8		2·8
L→R		5·8		3·6					3·8	
L Black proj.	9		7·4				5·4	4·8		5·4

Observer T.

	17. xii. 1928. 26 cm. disc					
R→L		3·8		4·4	3·4	
R Black proj.						6·2
L→R			3·8		5·2	2·6
L Black proj. 4·2						

The conclusion seems inescapable that somewhere an interaction is occurring between the retino-cerebral apparatuses of the two eyes, and that the formation of an image of a plain white screen on one retina is facilitating the early development of an after-image of a white object previously presented to the other eye; for, at the time when the $R \rightarrow L$ after-image is first seen, nothing but a uniformly dark field would be

visible with the right eye acting alone, and nothing but the white screen with the left eye acting alone. And it is difficult to believe that any sensory fusion, or binocular interference *in consciousness*, occurs prior to the appearance of the after-image, since the screen appears no less white than normally until, at the moment of the image's arrival, it suddenly becomes grey. The interaction must therefore occur at a sub-perceptual level.

It should be emphasized that the validity of this argument for interaction is not bound up with any particular theory of the seat and mode of causation of after-images. The shortening of the latency may or may not be due to the same factors as are responsible for the differences which we found to exist in Part I, between the latencies of black projection images and those of after-images projected on to a white screen presented to the eye (or eyes) on which the primary image of the disc had fallen.

It has been pointed out already that $R \rightarrow L$ and $L \rightarrow R$ images resemble uniocular *Black proj.* images in their general features. Like them, they sometimes failed to appear in our experiments when the fixation period was 15 sec. instead of the usual 30 sec., and second phases were of infrequent occurrence except with very large discs. With 26 cm. discs the first phase of the after-image appeared blue; the second black. Observer C. saw second phases more frequently, and the after-image tended to be more intense, in $R \rightarrow L$ than in $L \rightarrow R$ observations. Positives were occasionally seen before the negatives, especially by Observer H. Both of us found the images more distinct (and H. found them easier to time) than uniocular *Black proj.* images; it was extremely rarely that the after-image appeared as an incomplete disc.

It will be noticed that the figures recorded in this section show that in Observers C. and H. the latencies of $R \rightarrow L$ images are shorter than those of $L \rightarrow R$. This has been our invariable experience, and is doubtless ascribable to the same cause as the tendency to greater distinctness and longer duration of the former, which was noted in the last paragraph. It will also be remembered that the latencies of *R Black proj.* images were, in these observers, almost invariably shorter than those of *L Black proj.* *L Black proj.* images were also generally fainter, more often incomplete, and with large discs showed less trace of colour, than *R Black proj.* The explanation of these differences between *R* and *L* is probably to be found in the fact that both of us are in the habit of using our right eyes for such purposes as looking through a microscope, and hence of concentrating attention on the field seen with this eye, to the neglect of a different field presented to the other eye. (Vision is equally good with either eye.)

In conformity with this, Observer C., on one occasion when a 12 cm. disc was being fixated with the *L* eye (the *R* eye being covered), noticed that the whole primary image, except a small area round the fixation point, disappeared from view during the latter part of the fixation period. And on several occasions he has repeated the experiment described

by Schön and Mosso(13), of exposing one eye to an area of white screen, seen through a black tube, while keeping the other eye covered. The white field was then found to fade from view completely for three short periods within a minute of observation when the right eye was the covered eye, but no fading occurred when the conditions were reversed. (Incidentally, this last finding shows that simple binocular rivalry between the fields will not explain the observed darkening of the screen on the appearance of $L \rightarrow R$ images; the darkening must instead be attributed to rivalry between the white field seen with the R eye and the "black field *plus* after-image" seen with the L.)

Further evidence favouring the above interpretation is supplied by the experiments with Observers L. and T., both of whom have 6/6 visual acuity with each eye, and habitually use their left eyes for microscope work. Observer L. remarked spontaneously that, when the primary image was received by his right eye, the disc became blurred and almost faded at intervals during the fixation period, while no such effect occurred during fixation with the left eye. In three pairs of observations the $R \rightarrow L$ after-image appeared more than 1 sec. later than the $L \rightarrow R$. In Observer T., *R Black proj.* after-images with 22 cm. and 26 cm. discs showed a longer latency than did *L Black proj.*, but there was no detectable difference in latency between $R \rightarrow L$ and $L \rightarrow R$.

Part III. *Observations of the type* Bin.→R.

In a number of experiments we have studied the time-relations of the after-images which are seen when, after the white disc has been fixated with both eyes, one eye only is exposed to the white screen on which the after-image is to be projected. The results are interesting as confirming the conclusions reached in Part II, and show interaction between the processes tending on the one hand to produce a black projection image as a result of covering one eye at the end of the fixation period, and those tending on the other hand to give a white projection image from the eye which is kept open throughout the experiment.

In Observer H., with discs of 10 cm. and 26 cm. diameter, the after-image is seen after the same latent period as the ordinary white projection image, which it at first resembles in appearance (*i.e.* dark grey or black, with little or no halo). After being visible for a short time, this image is suddenly cut short, and is replaced by a typical black projection image with a strong halo and a bluish tint. Sometimes this transition is marked by the appearance of a positive image, inside which the second negative image develops. Two experiments of this kind are the following:

Observer H.

21. ix. 1928. 26 cm. disc

| | | |

Observer H (*contd.*).

29 ix. 1928. 26 cm. disc

R			2		2			
L Black proj.							3	3·2
Bin.→R	2·6		2*		2†			

* No halo; followed *c.* 3·6 by phase with halo.
† Like last; phase with halo *c.* 3·2.

Observer C. has found, in the three following experiments with a 26 cm. disc, that no image at all is visible at the usual time of appearance of an image projected on to a white field. Only later does an after-image appear, and it is, from the beginning, of the black projection type, *i.e.* it has a bright halo and the whole screen, with the exception of the halo and a small area round the fixation mark, darkens when it appears. The fact that an image of black projection type is later going to appear thus prevents the development of the image which, had the now covered eye not received the primary image of the disc, would have been plainly visible.

Observer C.

21. ix. 1928. 26 cm. disc

Bin.		2		2·4		2·2		
Bin.→R			3·4		3·4			
Bin.→L	2·6		3·4		2·6			
Bin. Black proj.							3·8	3·6

29. ix. 1928. 26 cm. disc

R		1·8		2·2		2·2		
L Black proj.							13·2	15·8
Bin.→R	3·4		3·6		3·6			

17. xii. 1928. 26 cm. disc

L	2·4		3·2		3·2			
R Black proj.		12		8·2		12·2		
Bin.→L			4·6		4·8			4·8

It is also evident from these figures that the same sort of interaction is at work that was noted in Part II. The *Bin.→L* after-images, although of black projection type, appear much earlier than do *R Black proj.* (or even *Bin. Black proj.*) images. This must mean that the arrival of the after-image, resulting from the previous stimulation of the covered eye, is being hastened by the stimulus which the image of the white screen supplies to the retina of the open eye.

This latter type of interaction is made still more evident in such observations as the following with a 4 cm. disc. The rate of development of the after-image was here so much accelerated for both observers that, although of undoubted black projection appearance and unmistakably different from the simple white field image, the *Bin.→L* image was actually timed earlier than the *L* (and hence, presumably, than *R* or

Bin.). In another experiment with a disc of this size, *Bin.*, *Bin.*→*R*, and *Bin.*→*L* all showed approximately the same latency. After-images of these small discs, projected on a white screen, are difficult to time exactly, owing to their slow steady rise(3); they are always very much earlier than the corresponding *Black proj.* images.

	Observer C.			Observer H.		
	29. ix. 1928. 4 cm. disc			29. ix. 1928. 4 cm. disc		
L	5·4	5·6		4·2	3·8	
R Black proj.			14·8			9
Bin.→L	4	4·6		3	2·8	
	Both of Black proj. type from beginning.			Both of Black proj. type from beginning.		

Discussion.

Until 1904, when Sherrington(14, 15) published a full account of his experiments with the binocular flicker lantern, it was commonly supposed that impulses originating from corresponding points in the two retinæ converge on a single common point in the sensorium. The site of the fusion of the twin paths from the eyes was variously placed either in the optic thalamus or in the occipital cortex, but some such sharing of a common central apparatus at a physiological (or apsychical) level was held to account for the singleness of the visual image excited from corresponding retinal areas.

Sherrington's ingenious experiments demonstrated the falsity of this assumption, and revealed a somewhat surprising degree of independence between the central apparatuses connected with the two eyes. So far, at any rate, as the qualities brightness and flicker were concerned, the retino-cerebral apparatus belonging to each eye was shown to develop its own sensual image with hardly a trace of interference from processes simultaneously occurring in the retino-cerebral apparatus of the other eye. The fusion of the two uniocular percepts, which had each been elaborated independently by their respective neuro-psychical mechanisms, to form a single binocular percept, appeared to be a psychological process, with no physiological basis of interaction between their respective paths below the level to which consciousness was adjunct. The reason why the binocular process is not amenable to analysis into its components by introspection must, then, be because these components are presented to consciousness as independently elaborated entities *simultaneously*, and not because of any fusion or interference between the apsychical or even sub-perceptual events arising from corresponding

retinal points. "It is much as though, of the left and right lantern images, each were seen by one of two observers, with similar vision, and as though the minds of the two observers were combined to a single mind" ((14), p. 56). "The...experiments...show that during binocular regard of an objective image each uniocular mechanism developes *independently*—at least as to steadiness of brightness and intensity of brightness—a sensual image of considerable completeness. The singleness of the binocular perception results from the combining of these elaborated uniocular sensations: it is the product therefore of a psychical synthesis that works with already elaborated sensations contemporaneously proceeding" ((14), p. 60). "The cerebral seats of right-eye and left-eye visual images are shown to be separate. Conductive paths no doubt interconnect them, but are shown to be unnecessary for visual unification of the two images" ((15), p. 383).

This theory only touches the fringe of the many problems presented by the highest form of binocular vision, viz. stereoscopic vision, but it obviously accounts satisfactorily for binocular rivalry and for simple cases of binocular fusion, and has the great merit of furnishing a ready clue to the interpretation of Fechner's "paradoxical experiment." Other considerations have led Snellen to somewhat similar conclusions, which have recently been thus expressed by one of his supporters: "Nous considérons donc les deux yeux comme deux organes tout-à-fait indépendants, dont la coopération doit être regardée comme une conséquence logique de l'attention; bien qu'en dernière instance ces deux organes soient fortement liés l'un à l'autre" ((6), p. 89). (Cf. also Helmholtz ((8), p. 407) and McDougall ((9), pp. 217–20).)

Sherrington's conception is of such fundamental importance, and is based on such a firm experimental foundation, that no evidence of interaction between the two uniocular mechanisms at an apsychical or physiological level can fail to be of interest as modifying, to a minor extent, the general conclusions outlined above. Sherrington's experiments themselves showed some trace of such interaction; when, for instance, alternations of light and darkness in the two uniocular images were arranged to be in opposite phase, flicker was found to be abolished at a very slightly lower critical frequency than when the flickerings were exactly in phase.

No further clear evidence of physiological interaction appears to have been forthcoming until 1923, when Allen(1) fatigued one eye with monochromatic spectral lights. He then found, by measurements of the critical frequency for extinction of flicker, that the brightness of certain

parts of the spectrum was enhanced for the other eye by some "reflex process."

The experiments described in the present paper provide additional information on this subject. In Part I of our results it was found that *Bin. Black proj.* after-images become visible after a shorter latent period than do *R Black proj.* or *L Black proj.* The uniocular mechanisms therefore reinforce one another at a subconscious level; for by no purely psychical combination of independently elaborated uniocular percepts could a binocularly aroused after-image become visible at a time when nothing but a homogeneous dark field would be seen with either eye acting alone. In Part II it was found that $R \rightarrow L$ after-images develop more rapidly than do *R Black proj.*, which they resemble in general appearance. The stimulation of the retina of the previously covered left eye by the image of the white screen, therefore, hastens the arrival of the after-image; and again a psychical synthesis is obviously inadequate to explain the result. A psychical fusion or rivalry of a rectangular white screen with a uniform dark field could not produce a negative after-image of a circular disc. In Part III interaction taking the same form as in Part II was demonstrated, and it was also found that, under certain conditions, the development of an after-image by the retino-cerebral apparatus of one eye might be prevented or delayed by events in the other eye, which were later going to produce an after-image of a different type.

We can hazard no guess as to the site or nature of the interaction in these cases. The mode of production of after-images is still so uncertain that it is impossible to say whether, for example, inhibition or summation or some more complicated process should be invoked to account for Part I and Part II of our results. But, though fully conscious both of the great limitations of any method involving the study of after-images, and of the reasonableness of the widespread prejudice against drawing important deductions from such experiments, we can see no alternative to interaction between the retino-cerebral apparatuses of the two eyes, at a sub-perceptual level in the brain, as the explanation of our results.

Summary.

1. After visual fixation of the centres of white circular discs, the time intervals which elapse before the appearance of black (negative) after-images have been measured under varying conditions.

2. After-images projected into complete darkness (*Black proj.*) have

a longer latent period than those projected on to a white screen. They also have a much more distinct halo and, with small discs, are more intensely black. With large discs they sometimes appeared coloured in our experiments.

3. Binocular and uniocular after-images were found to have much the same latency when projected on a white surface.

4. *Bin. Black proj.* images, on the other hand, appear earlier than *R Black proj.* or *L Black proj.* The latter are less distinct than the former, but are generally easy to time. In order to account for the early arrival of the binocular image, we must assume that the uniocular processes reinforce one another (or are otherwise interacting).

5. If the primary image is received by only one eye and the after-image is projected on a white screen presented only to the other eye ($R \rightarrow L$ and $L \rightarrow R$), the latent period is longer than when the same eye is used throughout the experiment. The after-image in the former case has a bright halo, and at the moment of its arrival the whole screen appears to darken and remains so until it fades. It is, therefore, of black projection type, and is in successful rivalry with the white field by which the now open eye is being stimulated.

6. The $R \rightarrow L$ image, however, appears earlier than the *R Black proj.* Its arrival is, therefore, hastened by the formation of an image of the white screen in the *L* eye, and this indicates interaction at a sub-perceptual level between the retino-cerebral apparatuses of the two eyes.

7. It is suggested that an explanation may be sought for certain differences between $R \rightarrow L$ and $L \rightarrow R$ images, and between *R Black proj.* and *L Black proj.* images, by reference to the habits of the observers in concentrating their attention on the field of view of one eye rather than on that of the other.

8. Further evidence of interaction is afforded by observations of the type *Bin.* $\rightarrow R$.

9. The evidence already adduced for interaction between the two uniocular mechanisms is discussed in its relation to Sherrington's experiments on binocular flicker.

Mr A. W. D. Leishman and Mr W. S. Tegner have kindly acted as observers in some of these experiments.

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THE HEAT PRODUCTION OF SMOOTH MUSCLE.

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Our knowledge of the physiology of muscular contraction is based mainly upon studies of vertebrate skeletal muscle. The study of a very different type of muscle might be expected to be of value (*a*) for the understanding of its special properties, and (*b*) to show which of the properties of skeletal muscle are common also to other types and may, therefore, be regarded as properties of muscular tissue in general. In the course of comparative studies on unstriated muscle(2) an easily obtainable and very favourable preparation was found in the retractor of the pharynx of the large edible snail (*Helix pomatia*): this muscle was used for all the experiments on heat production reported here.

Preparation. The muscle is a part of the columellar muscle. It is attached at one end to the axis of the shell and at the other to the ventral side of the pharynx. The shell is first removed, care being taken not to break the middle part of its axis, which is later used as an attachment for the muscle. After removing the front part of the mantle, which is very slimy, the body wall is cut dorsally from near the top up to the head. The genital organs are removed. If the stomach be then lifted with forceps, the pharynx and the retractor muscle attached to it beneath the œsophagus become visible. The pharynx is then cut off the body near the mouth. This should be done as quickly as possible since it involves cutting the connection of the muscle with the central nervous system, which forms a ring round the pharynx. Strong stimulation of the ganglia produces a vigorous contraction of the retractor muscle which persists for a long time, even after complete removal from the body. The axis of the shell with the muscle attached to it is cut off. Only as much of the axis is left as is necessary for the attachment. The other parts of the columellar muscle attached at the same place are removed as far as possible.

The axis is a narrow tube-like structure, and by pushing a thread or a wire through the opening an excellent attachment can be obtained. The other end of the muscle is fixed by means of the pharynx.

In preparing the muscle great care must be taken not to stimulate it, and to keep it entirely uninjured. This can easily be done, as it lies free in the body cavity and is barely connected to other tissues except at the two ends. Nevertheless, even after very careful preparation and much experience, the muscle is often obtained in a contracted state. Generally, however, it will relax after half an hour if immersed in snail's blood. This, therefore, is collected from the same animal before the dissection. One can easily obtain several c.c. of the blood by making a hole in the shell at the second turn on the side towards the top, where an artery is to be found. The blood flows out naturally as soon as this artery is cut: squeezing the animal will accelerate the flow.

It is important to keep the animals in perfect condition, otherwise the muscle preparation is very liable to go into long-continued contractions after a short stimulus. The animals seem to be in the best condition for the experiment in the winter. They should be kept in a refrigerator, and a few days before they are to be used should be brought into a warm room, kept moist, and fed with cabbage as soon as they creep out.

No salt solution was found as a satisfactory substitute for blood. The best results were obtained with a frogs' Ringer's solution containing three times the usual amount of calcium chloride.

Method. The method of heat measurement was that of A. V. Hill(8). Only a few modifications, which were required to adapt it to the new type of muscle, need be mentioned.

The muscle is fairly sensitive to stimulation and responds to single weak induction shocks. It is, however, not always certain that the heat produced by the current is quite negligible. Stimulation, therefore, was carried out by two electrodes lying close together above the thermopile. The stimulating current did not pass through the part of the muscle lying on the thermopile. A third electrode, below the thermopile, served for calibration. It was found that, using sufficiently strong stimuli, the mechanical response was the same whether the muscle was stimulated only at one end or by a current passing from one end to the other. No measurable effect of the heat produced by the stimulating current between the upper pair of electrodes could be detected at the thermopile, as was shown by tests with the dead muscle. The end attached to the columella was connected to the tension lever by a thread. The other

end was fixed below the thermopile by a curved pin pushed through the pharynx.

The calibration was carried out as described by Hill. Considerable difficulties arose, however, from the fact that it is very difficult to kill the muscle without causing it to alter its shape. By "electrocution" only the parts near the electrodes are killed: the middle parts still contract afterwards. Pure carbon dioxide, even when saturated with alcohol vapour, does not make the muscle sufficiently non-irritable. The method finally adopted was the following. The muscle was stimulated with a faradic current or a series of condenser discharges, beginning with weak stimuli and very gradually increasing them. Short periods of rest were interposed. If this process was continued for about an hour the response to a strong current was only about one-third of that of the fresh muscle. If, then, the chamber was filled with pure carbon dioxide, the muscle no longer responded to the alternating current used for calibration. It was not killed by this procedure: it recovered if left in air. That no appreciable heat, however, was produced actively by the muscle, under these conditions, was concluded from the fact that the heat liberated by the alternating current was exactly proportional to the square of that current as measured by the hot wire in the same circuit. With completely dead muscles it was ascertained that the result of the calibration is the same in carbon dioxide as in air, as was found by Hill.

Some difficulty was experienced at first, with the sensitive arrangement desirable for these experiments, in obtaining a constant zero. The influence of slow changes in the bath temperature could be largely eliminated in the following way. This influence appears to be chiefly due to heat radiation from the walls of the chamber. The thermopile, therefore, was adapted so that this radiation affects the hot and the cold junctions equally. This was done by varying the amount of wax on the cold junctions, removing some of it if warming of the bath caused a galvanometer deflection to the warm side, adding to it if the opposite effect was observed. With the thermopile first employed there is the disadvantage that it is less sensitive when there is little wax on the cold junctions, since their heat capacity is small and they are rather quickly warmed by the heat conducted from the hot junctions.

This disadvantage was avoided by winding the wire of the thermopile on a copper frame, shown in Fig. 1. The cold junctions lie close to a large heat capacity with a good heat conductivity, and the wires need only be covered with a thin sheath of wax. The cold junctions are not exactly

opposite to the warm junctions, more than two-thirds of each turn is silver-plated. This (*a*) diminishes the electrical resistance, and (*b*) delays the conduction of heat to the cold junctions, because the heat conductivity of constantan is so much less than that of silver. Both ends of the frame are covered by a copper plate, so that the interior of the thermopile is completely closed. The muscle side is covered by a very thin sheet of mica fixed by a thin layer of paraffin, in order to obtain a perfect and permanent insulation against water and electrical leaks.

Fig. 1. Cross-section of thermopile.

This very sensitive thermopile "settles down" in air and with no muscle on it in a quarter of an hour. With a muscle on the hot junctions, however, almost invariably a negative or positive deflection, sometimes a large one, persists for hours, and diminishes but slowly even when every precaution is taken to avoid leaks. It was finally found that the cause of this phenomenon was a difference between the vapour pressures (*a*) of the Ringer's solution at the bottom of the chamber, and (*b*) of the muscle. This deflection can be made positive or negative at will by using either diluted or concentrated Ringer's solution. Water will condense on the muscle in the first case, evaporate from the muscle in the second case, and thus give apparent heat production or absorption. The importance of this factor has already been realized by Hill(9). The zero becomes much more steady by introducing into the chamber a Ringer's solution of appropriate concentration, which must be found by trial. It is not possible, however, under these circumstances to measure the rate of resting heat production. To do so requires that the muscle should first be soaked in the Ringer's solution and brought into equilibrium with it, which is possible with frog's muscle, but, in the absence of a suitable solution, not with the muscle employed in the present investigation.

Some possible errors may be mentioned. (*a*) There is no distinct maximal response as in the skeletal muscle of a frog. If the stimulus be increased gradually the response increases more rapidly at first, and then more slowly over a considerable range. Strong stimulation must be avoided. It produces long-continued activity accompanied by heat production. This is the case, even if only a small part of the muscle be stimulated directly with excessive current. There is the possibility that not all the fibres respond to stimulation of moderate intensity. This, however, would hardly give an appreciable error in the heat measurement, because the muscle is so very thin, thinner indeed than the insulation,

that it allows a very rapid conduction of heat from any part of it into the thermopile. (*b*) Parts of the muscle near the stimulating electrodes might contract more strongly than those on the thermopile. One of my records of action current shows that the excitation produced by a weak but just adequate stimulus (slight mechanical response) applied at one end is not conducted to the other end, whereas that due to a stronger stimulus is. This condition, however, is rare. With stronger stimuli there is never a difference in the response, whether the whole muscle be stimulated or only one end. Repetition of such stimuli produces the same high maximum tension, which may be more than 5 kg. per sq. cm. of cross-section. Unequal tension in different parts would produce a deformation of the muscle which does not normally occur. The weak make shocks of a faradic current should be eliminated. (*c*) The most serious source of error is probably the occurrence of responses which outlast the stimulation. This can be avoided to a large extent by careful dissection, and by the use of moderate stimuli. Preparations which do not give a perfectly smooth relaxation curve after each response should be discarded.

The muscle has the form of a flat band, 4 to 6 cm. long, about 2 to 3 mm. broad, and about 0·3 mm. thick. It is uniform in cross-section and parallel-fibred. A detailed description of its mechanical response and its mechanical properties is to be given elsewhere (2). It is characteristic that the rise of tension is very rapid, the maximum being reached after 0·3 sec. in a twitch at room temperature. Relaxation, however, is very slow and lasts several seconds at least. Measurement of action current records shows that the wave of excitation spreads at a rate of 40 to 60 cm. per second. The contraction, therefore, of the whole muscle is practically instantaneous.

Snyder (13) has recently published a paper on the heat production of smooth muscle from the tortoise. His method seems to be open to many objections, and his actual observations (though to a less extent his conclusions) differ so much from those recorded here that a comparison of the results seems impossible.

Results.

Delayed heat. The galvanometer returns to zero much more slowly after the contraction of a live muscle than after the corresponding heating of a dead one, which shows that a considerable part of the heat is produced after the end of the mechanical response. The area of the galvanometer deflection curve for a contraction produced by 1 second stimulus was measured. It was assumed that the initial heat, the part which is

associated with the mechanical response, is measured by the maximum deflection of the galvanometer. A "control" heating of 2½ seconds was employed. For the same maximum deflection, then, the total area of the deflection-time curve, in the case of the contraction of the live muscle, divided by the area for the control heating of the dead muscle, is equal to the ratio of the total heat to the initial heat.

The method is correct only if the initial heat be produced within a short interval, otherwise the maximum deflection is not a true measure of it. It is necessary, therefore, to choose conditions in which relaxation is rapid. This is the case only in fresh muscle in perfect condition. The experiments were carried out at a fairly high temperature, 20–22° C. This assists rapid relaxation. The recording system, moreover, was made slow by employing thicker insulation, so that the maximum deflection was reached only in about 7 seconds. These precautions are sufficient to ensure that no appreciable amount of initial heat is produced after the maximum deflection is attained. A slight error may arise owing to the fact that the later part of the relaxation heat may not be measured quite accurately in the maximum deflection. There is, however, certainly very little heat produced in this last phase of relaxation (see below). Any error introduced in this way would tend to make the ratio of total to initial heat, as observed, too great, by allowing the initial heat to appear to be slightly smaller than it actually is.

The results of eleven experiments are given in Table I.

TABLE I. Ratio of total heat (in air) to initial heat, in snail's muscle.

Date, 1929	Ratio	Date, 1929	Ratio
16. ii.	2·2, 2·2, 2·2	11. iii.	1·8, 1·9, 1·9, 1·9
28. ii.	1·8, 1·8	14. iii.	1·9, 1·9
1. iii.	2·0, 2·1	18. iii.	1·8, 1·8
2. iii.	2·3, 2·3, 2·3	20. iii.	2·4, 2·4, 2·4, 2·4
3. iii.	2·25, 2·3	21. iii.	2·1
10. iii.	2·3, 2·4		

Mean value of ratio = 2·1.

The deflection curves are perfectly smooth (Fig. 2) and agree almost exactly in different experiments. The delayed heat starts at a high rate and is finished in 4 or 5 minutes, which is quicker than in frogs' muscle. It must be remembered that the actual amount of energy liberated in these contractions is very small, about 1500 g. cm. per g. The delayed heat lasts longer after long-continued contractions, and after the liberation of greater amounts of initial heat. It was also found that washing the muscle in Ringer's solution reduces the rate of recovery

heat production; the same is true for CO_2, even in fairly low concentrations (see below).

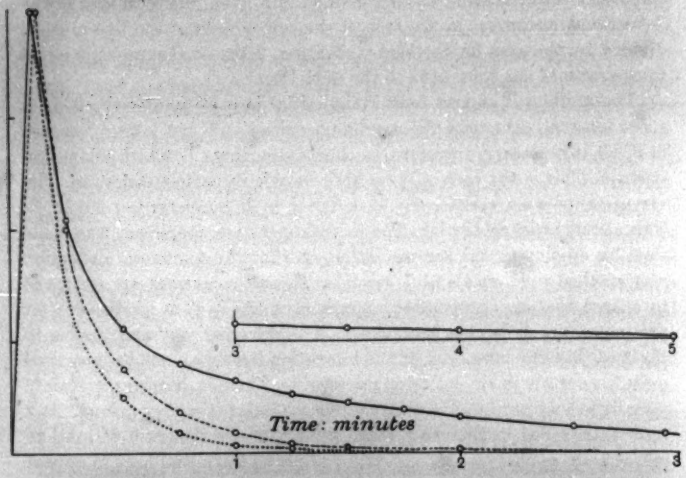

Fig. 2. Experiments of 2. iii. 1929. Galvanometer deflection curves: full line for contraction in air; broken line for contraction in nitrogen—CO_2 mixture; dotted line for control heating for 2·5 seconds. (Total heat)/(initial heat) = 2·3. Inset: continuation of curve in air.

The delayed heat, or at any rate the greater part of it, disappears in the absence of oxygen. The muscle was deprived of oxygen by placing it in nitrogen, purified by the method of Kautsky and Thiele(10). This has the peculiar effect of making it extremely irritable. After a weak short stimulus it usually gives a strong contraction lasting for several minutes. After being in pure nitrogen for some time it often starts contracting spontaneously. To avoid this the nitrogen was mixed with carbon dioxide (about 25 p.c.) which acts as a weak narcotic. This, however, has the disadvantage of making the muscle extremely slow, relaxing completely only after several minutes. The maximum galvanometer deflection, therefore, cannot be expected to represent the initial heat with any accuracy. Even so, however, the deflection of the galvanometer invariably comes back more quickly than in air (Fig. 2). In nitrogen the area of the deflection curve is generally 30 to 40 p.c. greater than that of a control heating curve of the same maximum deflection.

In one case in which the relaxation was relatively quick, it was only 11 p.c. greater. It is unnecessary to give these results in detail, as they cannot be regarded as quantitative.

We may conclude that, in the snail muscle, as in the skeletal muscle of frog or tortoise, the heat consists of two parts, the initial heat, which is anaerobic, and the delayed heat, which is produced only in the presence of oxygen. The ratio of these to one another is the same as in the frog's sartorius.

The isometric heat coefficient. The energy used for producing tension has been expressed by Hill in the quotient H/Tl, where H is the initial heat, T the maximum tension, and l the length. This has been determined for snail's muscle for different durations of the stimulus. The initial heat was measured by the maximum deflection of the galvanometer: the same precautions, therefore, were necessary as before, in order to be sure that the initial heat was produced within a sufficiently short time. If we plot H/Tl against duration of stimulus we obtain a straight line (Fig. 3):

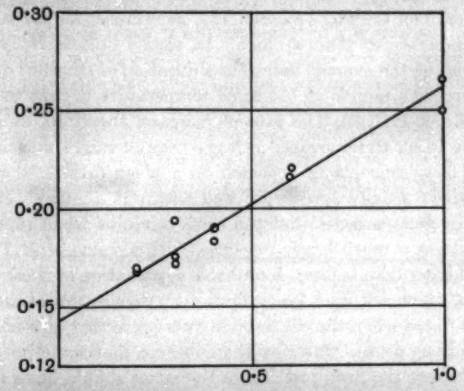

Fig. 3. Experiment of 17. ii. 1929. H/Tl for different durations of stimulus. The points shown are single observations. Time in seconds.

this relation, which is the same as for the frog's sartorius, has been discussed by Hartree and Hill(5). The heat can be regarded as consisting of two parts. One part is related to the production of the tension: this is constant and is given by the value of H/Tl for short durations of the stimulus. The other part is related to the maintenance of tension

already produced: this is proportional to the duration of the stimulus. We obtain therefore the equation

$$H/Tl = a + bt,$$

where a and b are constants, and t the duration of the stimulus. For the frog's sartorius a is 0·16, and has been found to be of the same order of magnitude for other kinds of vertebrate skeletal muscle(7). It is interesting that it has almost the same value for snail's muscle. The values obtained in nine experiments were

0·15, 0·15, 0·11, 0·14, 0·15, 0·17, 0·14, 0·15, 0·12; mean = 0·142.

The values must be extrapolated to zero time as shown in Fig. 3, since it was not possible to apply stimuli shorter than 0·15 sec. at the low frequency of stimulation used in the present experiments.

The constant b determines the slope of the line. It is very different for different muscles. Hill has shown that it depends on the time relations of contraction. Less energy is spent in maintaining a tension in slow than in quick muscles: b therefore is much smaller for the tortoise's than for the frog's muscle. It is also smaller for the same muscle at low temperatures than at high. In snail's muscle H/Tl has been found to rise on the average to 0·29 at a duration of stimulus of 1 second, whereas in frog's muscle at the same temperature it rises to about 1·0 (Hartree and Hill(5)). The rate of increase, therefore, of H/Tl with time is five to six times greater in frog's than in snail's muscle.

If the measurements be repeated on a given muscle, the values of H/Tl obtained usually agree very well with one another. It happens, however, in some muscles that the same stimulus when repeated suddenly produces a much larger response, with a value of H/Tl as much as 40 p.c. higher than before. A probable explanation of these occasional differences, which are much larger than the possible error of the measurement, was found when the contractions were recorded at a higher speed of the revolving drum. One may then observe that sometimes a second response follows the first, just before the latter should have reached its maximum. This is shown by a distinct discontinuity of the tension record, and by a late maximum of the curve. The great sensitivity of the muscle to mechanical stimuli may be the cause, slight movements of the muscles over the electrodes producing mechanical irritation during the contraction, and a resulting second response. This would have the same effect as a greater duration of stimulus, and would explain the higher tension and the greater value of H/Tl.

The economy of maintaining tension. It is characteristic of the type

of muscle studied that it can maintain a tension very economically. This is probably its most important difference from skeletal muscle. The energy required for maintaining tension, therefore, is in this case of special significance.

The tension maintained in a contraction is measured by the "tension-time" (Hartree and Hill(5)), that is, by the area of the tension-time curve $\int T dt$, where T is the tension, t the time. The efficiency in maintaining tension then is expressed by the quotient $\frac{\int T dt}{H}$. It is independent of the mass of the muscle, since both the tension and the heat are proportional to this. This quotient may be called the "coefficient of economy of maintaining tension," or, more shortly, the "economy." It has the dimension of time, which indicates that it depends on the time relations of the processes concerned. By H is meant only the initial heat in this connection.

Accurate determinations of the "economy" are difficult, because it changes fairly rapidly during a contraction. To avoid this only short contractions with small expenditures of energy must be used. The "economy" of fresh muscles was measured in a number of experiments in two different ways: firstly, a short tetanus was produced by stimulating for 0·1 to 1·0 second; the initial heat was measured by the maximum deflection; secondly, after complete recovery it was stimulated for 1 minute by one shock every 2 to 3 seconds and the total heat determined by measuring the area of the deflection-time curve. The initial heat of this contraction was taken as half the total heat. The results of a few experiments are given in Table II.

TABLE II. Coefficient of economy of maintaining tension: snail's muscle.

Date 1929	Stimulus (sec.)	Tension-time (g. sec.)	Heat (g. cm.)	Half relaxation (sec.)	"Economy" (sec.)
27. iii.	0·8	43·2	8·35*	1·5	12·0
	1·0	37·2	7·82*	1·5	11·4
	60	228	72†	1·8	14·4
4. vi.	0·1	24·6	2·15*	2·5	26·0
	60	229	33·0†	2·9	32·0
6. vi.	0·5	40·7	11·7*	1·2	8·2
	0·6	58·8	10·25*	1·6	13·0
	60	342	82†	2·3	19·2
17. vi.	1	37·8	5·83*	3·0	16·8
	60	170	42·6†	4·9	21·0
18. vi.	0·6	38·4	5·5*	3·0	18·6
	60	580	121†	6·5	25·0

* From maximum deflection. † From area.

Hartree and Hill(5) have pointed out, and shown experimentally, that in skeletal muscle the "economy" depends on the time relations of the mechanical response to a single stimulus; the slower the muscle the more tension-time is produced with the same expenditure of energy. To compare the time relations in the several muscles shown in Table II, the time required for the tension to fall to half its maximum value is given.

In agreement with its slower time relations the "economy" of the snail's muscle is found to be considerably higher than that of the frog's muscle. Values for the "economy" of the frog's sartorius are given by Hartree and Hill(5) in a graph showing the relation of heat and tension-time for different durations of stimulus and different temperatures. At 15° the "economy" for short stimuli (up to 0·2 sec.) is about 0·4. The higher values for longer stimuli may be partly due to fatigue. At 14° the tension falls during relaxation to half its maximum value in about 0·065 sec., as can be read off from curves given by the same authors. We will assume the value 0·060 for 15° C. In the experiment of 27. iii. 29 in Table II, the time to half relaxation was 1·5 sec. for the fresh muscle, which is 25 times as great as the value just given for the frog. The "economy" is 12/0·4 = 30 times greater. Other experiments give similar results. The comparison is of course only rough, but it shows that the values are of the order which might have been expected from the difference of time relations. There is, therefore, in this connection no difference of principle, but only of time relations, between muscles of the snail and of the frog.

In Table II the "economy" is found to be the same, as closely as could be expected, whichever method of measurement was adopted and with different durations and frequencies of stimulation. Such differences as occur may be partly due to a recovery heat coefficient other than 2, but chiefly to a change in the condition of the muscle due to previous stimulation. This is indicated by the change of the speed of relaxation: the muscle is slower at the end of a long contraction and the "economy" correspondingly greater.

This progressive change in "economy" with stimulation is a phenomenon of special interest. If the muscle be stimulated repeatedly it produces the tension with about the same amount of energy, but relaxation becomes slower and "economy" greater. In the experiments shown in Fig. 4 the muscle first gave the contraction *A* with a stimulus lasting 0·35 sec. This was repeated several times and then contraction *B* was obtained with a stimulus lasting 0·4 sec. The response of the galvano-

meter recording the heat production was recorded photographically as shown in the upper curves *A* and *B*, and a control curve was obtained

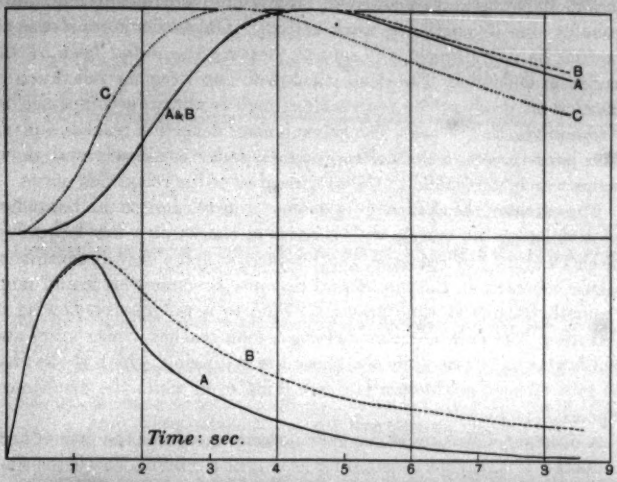

Fig. 4. Experiment of 15. vi. 1929, at 14·4° C. Lower curves, tension records of two contractions, *A* (full line) that of fresh muscle, *B* (broken line) after a period of exercise. Upper curves, galvanometer deflection records for the two contractions *A* and *B* shown below, and for 0·3 sec. heating (*C*).

by heating the muscle for 0·3 second (curve *C*). The galvanometer deflection curves for the contractions have their maxima 1·6 sec. after the control, and after their maxima the curves fall off almost as rapidly as the control. This shows that by far the largest part of the initial heat is produced within less than 2 seconds, and that the heat produced during the later stages of relaxation is small. This was the case, although relaxation was rather slow in both cases and the temperature low (14·4° C.). The curves for both contractions fall off in very nearly the same way. This shows that insufficient heat was produced in the later stages to account for the much slower relaxation in contraction *B*, thus excluding the possibility that the change in time relations is simply the effect of further weak activity of the muscle during relaxation. An accurate analysis of the curves by the method of Hartree and Hill[4] would show this more clearly, but a quicker recording system than that used here would be required.

There are, however, cases of slow contractions in which the galvanometer deflection curves fall off appreciably more slowly than the control curves. A considerable amount of heat is produced during relaxation, probably due to continued weak activity. Only, therefore, if the relaxation be fairly quick is it safe to measure the initial heat by the maximum deflection. The slow, incomplete and irregular relaxation of contractions produced by too strong stimuli is always accompanied by a large production of heat. The galvanometer deflection reaches a maximum later than in a normal contraction, falls very slowly and shows humps which correspond to the irregularities of the relaxation curve.

The extent of the change in "economy" due to exercise can be studied by stimulating the muscle with a series of shocks at a low frequency, one shock every 2 to 3 seconds. This produces at first a very discontinuous tetanic contraction, the mechanical response becoming more and more a smooth tetanus as time goes on, owing to a progressive slowing of relaxation. The galvanometer deflection soon reaches a maximum and then begins to return while the tension is still rising, which shows that the rate of heat production is diminishing even while the mechanical response is increasing.

A constant deflection of the galvanometer measures the rate of heat production if it remains constant for as long as a minute. This is the time necessary for the thermopile to get into a steady state, heat production being balanced by heat loss. During the first minute of the contraction, however, it is no measure of the rate of energy expenditure for maintaining tension, for (*a*) the muscle has not only to maintain but also to produce tension; this latter part is not quite negligible even for a fairly slow rise of tension; and (*b*) the recovery heat production will occur only a certain time after the beginning of the contraction. I have therefore measured the "economy" of the fresh muscle as described above, using contractions of short duration and measuring the total tension-time produced. The total energy liberated in such a contraction is small and the condition of the muscle may be regarded as constant during the short period involved.

The tension and the rate of heat production become approximately constant after the muscle has been stimulated at a low frequency for several minutes, and the muscle can be kept in practically a constant condition for at least 1 hour. It is justifiable, therefore, to assume that the muscle does not run into oxygen debt. This is shown also by the fact that after the end of the stimulus the galvanometer deflection comes back to about half during the first minute of relaxation and returns to

zero after about 15 minutes. The constant deflection of the galvanometer, therefore, measures the rate of total energy expenditure in maintaining tension, *i.e.* including recovery heat. The results of two experiments are given in detail in Table III.

TABLE III. "Economy" of maintaining tension in long contractions: snail's muscle.

Exp. 27. iii. 29. 20·2° C.

1 sec. tetanus, tension-time 37·2 g. sec., heat 7·82 g. cm., "economy" 11·4
60 sec. „ „ 228 g. sec., „ 72 g. cm., „ 14·4

Long tetanus, maximum deflection 425 mm., reached in 3 min. 15 sec., tension 12·5 g.

After 7 min., tension 13 g., deflection 140, "economy" 87
„ 13 min., „ 15·5 g., „ 201, „ 70
„ 19 min., „ 13·8 g., „ 181, „ 72

Rest for 20 min.

Long tetanus, maximum deflection 515 mm., reached in 2 min. 15 sec., tension 15·5 g.

After 7 min., tension 17 g., deflection 180, "economy" 89
„ 10 min., „ 14·1 g., „ 208, „ 66
„ 16 min., „ 10·5 g., „ 112, „ 88

Rest for 20 min. The same procedure repeated a third time gave the same result.

Exp. 6. vi. 29. 22° C.

0·5 sec. tetanus, tension-time 41 g. sec., heat 11·7 g. cm., "economy" 8·2*
60 sec. „ „ 342 g. sec., „ 82 g. cm., „ 19·2

Long tetanus, maximum deflection 475 mm., reached in 3 min. 45 sec., tension 20 g.

After 6 min., tension 23 g., deflection 231 mm., "economy" 72
„ 18 min., „ 18·5 g., „ 160 mm., „ 87

Relaxation, rest for 1 hour.

Long tetanus, maximum deflection 660 mm., reached after 1 min. 30 sec., tension 29 g.

After 4 min., tension 29 g., deflection 302 mm., "economy" 68
„ 9 min., „ 15·7 g., „ 108 mm., „ 102
„ 11 min., „ 13·5 g., „ 81 mm., „ 127

* Similar short contractions are interposed before the next longer contraction.

In all the experiments moderate exercise for a few minutes produced an increase in "economy" of six to ten times, and on occasions even as high as fifteen times. This change is not connected in any way with signs of deterioration of the muscle. Contraction may continue for some hours, and relaxation may in the end be many times slower. The difference in mechanical response between fresh muscle and muscle after exercise is illustrated in Fig. 5. The muscle stimulated by one shock every 3 seconds gave at first a very incomplete tetanus, but at the end a perfectly smooth contraction. The change is reversible. After 15 to 30 minutes' rest relaxation is very nearly, if not quite, as quick as at the beginning of the experiment, and the "economy" is low. The observation, therefore, can be repeated several times on the same muscle, as was done in the experiments given in Table III.

Another factor which has nearly as great an influence on "economy," under physiological conditions, as exercise is carbon dioxide tension.

The muscle chamber was filled with different mixtures of CO_2 and air, starting with low concentrations of CO_2. Short tetani only (1·0 sec.)

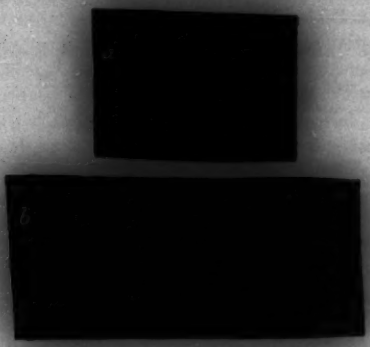

Fig. 5. Experiment of 6. vi. 1929, at 22° C. Tension records. The muscle was stimulated by one shock every 3 seconds; *a*, response of fresh muscle; *b*, after 10 minutes of exercise. Stimulus discontinued in middle of *a* and *b*. Time in 6 sec. units. 1 mm. = 1·1 g. tension.

were used to exclude the possibility of changes in the condition of the muscle due to exercise. The total heat was measured from the area of the deflection-time curve. Between every pair of contractions there was a pause of at least 10 minutes, which allowed complete recovery. At the end the chamber was again filled with air, and new measurements made to see whether the condition of the muscle had altered irreversibly. The slightly diminished oxygen concentration in the chamber in the CO_2 experiments had hardly any appreciable effect on the muscle. Some tests were made with a mixture of 5 p.c. CO_2 and oxygen, which gave exactly the same effect as the same concentration of CO_2 in air.

The results were very consistent. If "economy" be plotted against CO_2 tension a relation is found which is approximately linear, but increasing rather more steeply at the beginning. The results of two experiments are shown in Fig. 6. Table IV gives the results of all the experiments made (except preliminary observations).

The magnitude of the change of "economy" due to CO_2 varies in different experiments. At a CO_2 concentration of 3 p.c. it rises 2·8 to 6·6 times, with 6 p.c. CO_2 5·2 to 9·9 times. The effect is completely reversible. The last value obtained in air is generally slightly lower than

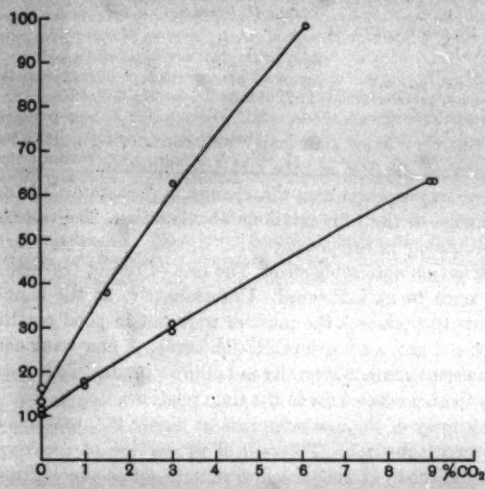

Fig. 6. "Economy" of maintaining contraction at different partial pressures of CO_2 in air. Upper line, experiment of 20. viii. 1929; lower line, experiment of 12. viii. 1929.

TABLE IV. Effect of CO_2 on the "economy" of maintaining tension: snail's muscle.

Exp. 12. viii. 29. 18·6° C.					
CO_2 (p.c.)	0	1	3	9	0
Heat (g. cm.)	37	40	45	40	68
Tension-time (g. sec.)	64	115	216	400	113
"Economy," absolute	11	18·4	31	64	10·7
"Economy," relative	1	1·7	2·8	5·8	0·98
Exp. 17. viii. 29. 19° C.					
CO_2 (p.c.)	0	1·5	3	6	0
Heat (g. cm.)	43·5	42·5	69	56	61
Tension-time (g. sec.)	67·5	161	350	465	75
"Economy," absolute	13·5	33	59	70	10·6
"Economy," relative	1	2·4	3·3	5·2	0·8
Exp. 19. viii. 29. 16° C.					
CO_2 (p.c.)	0	1·5	3	6	0
Heat (g. cm.)	51·8	36·3	35·9	53·6	51·3
Tension-time (g. sec.)	114	183	310	724	92
"Economy," absolute	16·3	38	63	100	13·3
"Economy," relative	1	2·3	4·0	6·1	0·8
Exp. 20. viii. 29. 16° C.					
CO_2 (p.c.)	0	1·5	3	6	0
Heat (g. cm.)	44·5	43·5	55	55·6	28
Tension-time (g. sec.)	55	143	415	680	39·2
"Economy," absolute	8·2	25	54	81	10
"Economy," relative	1	3·1	6·6	9·9	1·2

TABLE IV (*contd.*).

Exp. 26. viii. 29. 19·5° C.					
CO_2 (p.c.)	0	1·5	3	6	0
Heat (g. cm.)	30·6	43·2	33·5	40·7	48·5
Tension-time (g. cm.)	69·5	317	458	842	107·5
"Economy," absolute	11·7	38	67	107	11·4
"Economy," relative	1	3·2	5·7	9·2	0·98

the first, but they agree sufficiently well considering that 3 to 4 hours elapsed between the first and the last determination.

In these experiments also the change in "economy" is associated with a change in the time relations of relaxation. The muscle relaxes much more slowly in the presence of CO_2; even concentrations of 0·5 to 1 p.c. CO_2 have a noticeable effect. The rate of rise of tension, however, does not seem to be influenced. Unfortunately, at the time of these experiments (September), the muscles were not in good condition, and usually did not give a smooth relaxation curve. A new weak contraction often set in some seconds after the end of the stimulus. It is not possible, therefore, to give exact data to the time relations.

The accuracy of the measurements at higher CO_2 tensions is diminished by two factors: (*a*) The rate of production of recovery heat is greatly diminished. During the first minutes after the contraction the deflection of the galvanometer comes back more quickly with the muscle in CO_2 mixtures than with the muscle in air, although relaxation may be much slower: in the end, however, the galvanometer returns more slowly. This means that the recovery heat is spread over a longer period and is, therefore, more difficult to measure. The same effect has been noted for the frog's sartorius by Hartree and Hill(6). They found the rate of recovery heat production to be diminished to one-half in the presence of 25 p.c. CO_2. The effect is apparently much greater with the snail's muscle. It is noticeable at 6 p.c. CO_2 and considerable at 9 p.c. It cannot be ascribed simply to a slightly smaller oxygen tension in the gas mixture, but must be regarded, as in frog's muscle, as due to a diminution in the rate of oxidation by the increase of acidity. (*b*) A small residual tension may often be noticed for many minutes at higher CO_2 concentrations, due apparently to continued weak activity of the muscle. Under these circumstances the determination of the area of the tension-time curve is less exact. The heat associated with this continued activity of the muscle is of course included in the total heat. The possible errors which arise from (*a*) and (*b*) are certainly small at the low CO_2 concentrations used here, as compared with the magnitude of the changes produced by the CO_2. At higher concentrations, however, quantitative experiments have not been made for the reasons given.

An attempt has been made to determine the carbon dioxide dissociation curve for the snail's muscle, which seemed to be of interest in connection with the great effect of CO_2 on this muscle. The method used was that employed by Fenn(3) for the tissues of the frog. A muscle is brought into equilibrium with various concentrations of CO_2 in one of the chambers of a differential micro-respirometer. The combined CO_2 is then liberated by dropping sulphuric acid on to the muscle. Unfortunately the values obtained on different days do not agree sufficiently to give a smooth dissociation curve. This is probably due to the very variable water content of the animals employed. More constant conditions would probably give more consistent results. The values obtained, however, are of interest because they show the very large carbon dioxide combining power of the snail's muscle. If left in air the muscle contained 60 to 133 volumes of CO_2 p.c., and in 6 p.c. CO_2 it contained 140 to 180 volumes p.c. This is many times more than found by Fenn and by Stella for frog's muscle, and considerably more than has been found for vertebrate blood. The high CO_2 combining capacity may be connected with the great effect of CO_2 described above.

Discussion.

Smooth muscles differ from skeletal muscles mainly in two respects. (*a*) They are able to maintain a tension for a very long time with a very small expenditure of energy (see *e.g.* Parnas(11), Bethe(1)). (*b*) Their properties vary very much from one set of conditions to another. The differences are so great that it has been found difficult to apply to smooth muscle the principles established for skeletal muscle, and it has been doubted whether the mechanism of contraction is the same in the two cases.

A closer study, however, of the mechanical response of two smooth muscles, which will be published separately in detail(2), has shown that the differences are not so great as is often assumed. After every isometric contraction there is a relaxation, which differs from that of skeletal muscle only in its time relations. Under certain conditions the response may be as rapid as that of slow skeletal muscles, and the similarity between both kinds of muscles is then most striking. One might be tempted to think that the snail's muscle is not typical of smooth muscle in its physiological properties. The same time relations, however, of the contractions have been found also for the smooth part of the adductor muscle of *Pecten*, which has always been regarded as a typical smooth muscle. It should be noted that the responses of these muscles can be successfully studied only if they are in perfect condition. It is not possible to draw definite conclusions concerning the time relations of the contraction from experiments in which it is uncertain whether the muscle is still active at the end of stimulation, or in which the fibres do not contract simultaneously. Failure to obtain muscles in good condition may be one reason for the inconsistent results of earlier experiments.

In addition to the similarity of mechanical response, the myothermic experiments described here give results which in important points agree surprisingly closely with those obtained from the frog's sartorius. The isometric heat coefficient for short contractions, and the ratio of total to initial heat in the presence of oxygen, are practically the same. The fact that these values agree, for two muscles so different in their structure and taken from animals so different in their organization as the frog and the snail, may be regarded as a new proof of the fundamental significance of these values for muscular tissue in general. The present experiments have thrown light also on the question of the great economy of smooth muscle. They provide no evidence that tension can be maintained without heat production. The muscle sometimes gives long-continued contractions after a short stimulus, or it may contract spontaneously. In either event there is a production of heat which seems to be as large as that occurring when the muscle is stimulated. A number of authors have adopted the view that the great economy is due to the slow time relations of the response to a single shock. Hill confirmed this view by showing that slow muscles, like those of the tortoise, are more economical than fast muscles like those of the frog, and that the same muscle becomes more economical if its time relations are made slower, *e.g.* by lowering the temperature. The same standpoint has been adopted and developed in detail by Ritchie (12).

The snail's muscle may be fairly quick and relatively uneconomical after a period of rest. Moderate exercise, however, may make it much slower and fifteen times more economical. As the result of exercise alone the economy may rise to values of more than 100 in absolute units. One may calculate that the isolated muscle could maintain a tension of 12 g. for one day, *i.e.* 1·2 kg. per sq. cm. of cross-section, by supposing that the glycogen content of the muscle is the same as that in the frog (1 p.c.) and that this is the source of energy. It is true that some muscles, like the adductor muscles of certain Lamellibranchs, are probably still more economical, as judged by the time during which they can keep their shells closed. Under more normal circumstances, however, the change of economy might be even greater than in the case considered. I refer especially to the effect of CO_2, which may be one of the main factors determining the economy in the normal muscle. The necessity of maintaining tension for a considerable time arises particularly when the snail has retracted into its shell, or in the case of Lamellibranchs when the animal has closed its shell. The CO_2 tension may be particularly high under such conditions, and the concentrations used in the present experiments are probably still within the physiological range.

The change of time relations of the contraction due to exercise is not peculiar to the type of muscle studied here: it is probably of the same nature as that associated with fatigue in skeletal muscle. Hill has shown that H/Tl for a single shock is not changed in a moderate condition of fatigue. For the same maximum tension, however, the tension-time, and therefore the economy, are increased because the contraction is slower. This change has been studied in detail by Bronk and has been described in the last number of this *Journal*. Frogs' sartorii may become about three times more economical after severe exercise, and in fatigue. The change is probably small under physiological conditions. In the normal animal the importance of this change is certainly much greater in the case of the snail, not only because it is considerably greater but also because it occurs after very moderate exercise and relatively small expenditure of energy, long before any signs of exhaustion are apparent.

It seems likely that the change after exercise, as well as that under the influence of CO_2, is due to the same factor—an increase of acidity inside the muscle. Other factors, however, probably also are involved. The adductor muscle of *Pecten*(2) can relax in the intact animal very quickly, in less than 1 second, after being contracted for a considerable time. We can explain this fact only by supposing that a change of the time relations of relaxation, in a direction opposite to that resulting from exercise or CO_2, can occur under nervous influence. The question would seem to be of peculiar importance in the physiology of this muscle.

It seems, then, that the muscle can adapt the time relations of its mechanical response within rather wide limits to varying requirements. These changes in time relations will probably make more intelligible many of the properties of smooth muscle.

Summary.

The energetics of the retractor of the pharynx of the edible snail (*Helix pomatia*) have been studied with A. V. Hill's myothermic methods. The main results are as follows:

1. For short durations of stimulus H/Tl has an average value of 0·14, practically the same as for the frog's sartorius. It increases in a linear manner with the duration of the stimulus.

2. The ratio of total to initial heat in the presence of oxygen is about 2.1. The delayed heat is largely, probably completely, absent in the absence of oxygen.

3. The "coefficient of economy in maintaining tension" is expressed by the ratio $\int Tl dt/H$, where T is tension, t time, l length of muscle,

H initial heat. It is much greater than in the frog. It increases considerably after moderate exercise, in some experiments as much as fifteen times. This change is due to an alteration in the time relations of relaxation.

4. A considerable increase in "economy" (several fold) is effected by CO_2 in quite low concentrations.

5. No evidence has been found for the idea that tension may be maintained without heat production. The great "economy" of the muscle under certain circumstances can be explained by the slow time relations of the mechanical response.

I am greatly indebted to Prof. A. V. Hill for his kind interest in this work, for his criticism, and for his many valuable suggestions. My thanks also are due to Prof. W. O. Fenn, in whose laboratory I continued the work, for the generous hospitality which I enjoyed with him, and for his advice and instruction in the use of his methods. The work has been carried out during the tenure of a fellowship of the Rockefeller Foundation, and as a visiting member of the School of Medicine, Rochester, N.Y.

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ON THE SYNERGISM BETWEEN ŒSTRIN AND OXYTOCIN.

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I. Introduction.

There is ample evidence (discussed by Marshall(10)) to show that parturition takes place when a particular stage of the ovarian cycle is reached. Thus phenomena normally associated with parturition may occur when a similar ovarian stage is reached at the end of pseudo-pregnancy, while experimental acceleration(11) or retardation(14) of the ovarian cycle during pregnancy leads to premature or postponed parturition. This correlation might suggest that, at the end of pregnancy, the ovary produces some internal secretion acting directly on the uterus to cause the activity resulting in parturition. Such a possibility is supported by the fact that the spontaneous contractility of the uterus shows cyclic variation in intensity; but, though œstrin(2) may have the power of causing slight uterine contraction, it has not been possible to show that the ovary has any direct action on uterine contraction to the degree required to produce parturition.

In the circumstances it is natural to consider what part, if any, may be played by the oxytocic principle of the posterior pituitary body. Two alternate or complementary theories may be evolved:

(*a*) That the ovary in the pre-parturition stage produces some secretion to stimulate the posterior pituitary, which in turn stimulates the uterus.

(*b*) That the sensitivity of the uterus to oxytocin undergoes cyclic variation, in correlation with the ovarian cycle, the maximum sensitivity being reached at the time of parturition.

Dixon and Marshall(4) were able to give some experimental confirmation to the former view, but, more recently, considerable support

has been forthcoming for the latter. Knaus(5) found that posterior pituitary preparations interrupted pregnancy in the rabbit only during the last stages, and only then as the result of fœtal death due to crushing. Later, the same author showed by carefully controlled experiments that the isolated rabbit uterus was practically insensitive to the oxytocic principle during the greater part of pregnancy, a condition shown to be due to the action of the corpus luteum of pregnancy. More recently, Bourne and Burn(2) found that œstrin had an opposite effect on the uterine response to oxytocin, *i.e.* a sensitizing effect. Since normal parturition is preceded by the atrophy of the corpus luteum and, in certain species, at any rate, by the reassertion of the œstrus-producing stimulus, these experiments provided the nucleus of a theory of the initiation of parturition, and also offered some explanation of the fact that abortion may be produced by the injection of œstrin and also by the removal of the corpora lutea.

The experiments recorded in the present paper were concerned with (*a*) the action of the total posterior pituitary extract ("pituitrin") as compared with that of the separated oxytocic principle, (*b*) the conditions required to produce abortion with the present comparatively pure "water-soluble" œstrin, and (*c*) whether any synergism between œstrin and oxytocin could be demonstrated on the intact pregnant animal.

II. Material and technique.

Experimental animals. When the work was originally begun, it was hoped to be able to carry out some experiments on a large mammal, in which it would be possible to make assays of the oxytocic activity of the cerebral spinal fluid. In conjunction with Prof. E. B. Verney and Dr F. W. Winton, some initial experiments were performed on dogs and goats, but the difficulties of obtaining and housing the animals made it necessary to abandon this aspect of the work. For the experiments described in the present paper mice were used on account of being available in large numbers and being easy to obtain pregnant. They had, however, the disadvantage that pregnancy lasts 19 days only and cannot be detected with certainty before 11 days. In all the mice used for the experiments described, copulation was dated, and the presence of fœtuses confirmed at 11 days by the "placental sign" and palpation. Detection of œstrus, copulation, etc., was carried out by the routine methods.

Source of œstrin. The œstrin used was prepared from urine of pregnancy by Mr G. F. Marrian by methods already described by him(6).

Two different batches, one containing 40 m.u. per c.c. and the other 100 m.u. per c.c. were used.

Posterior pituitary preparations. When the work was commenced in 1927 Burroughs and Wellcome's "pituitrin," containing both the oxytocic and pressor principles, was used. Later, however, the separated oxytocin and vasopressin, in the form of Parke Davis' "pitocin" and "pitressin" became available. All extracts were injected subcutaneously.

Criteria of positive result. In investigating the mechanism of parturition, it is of importance to consider carefully the exact manner in which pregnancy is brought to an experimental termination. The chief ways in which pregnancy may terminate prematurely in the mouse are:

(*a*) *Abortion of live fœtuses.* This is clearly an essential criterion of real simulation of the act of parturition. Such abortion may take place 24 to 48 hours after the end of treatment, as when due to ovariectomy or occasionally injection of œstrin, or the effect may be immediate (1 to 6 hours) as when caused by injection of oxytocin at the end of a series of œstrin injections. When induced sufficiently late in pregnancy this type of abortion is indistinguishable from normal parturition.

(*b*) *Fœtal death* in utero. This may be due (in the experiments under review) to disturbance of the vascular connections, or to pressure set up by abnormal uterine contraction. The results of fœtal death may be (i) abortion of the dead fœtuses in 36 to 48 hours, (ii) reabsorption by the mother, especially in the early stages of pregnancy, (iii) occasionally "mummification" of the fœtuses, leading to retention in the uterus for long periods.

In Tables V and VI "immediate abortion" refers to the delivery of live fœtuses within 1 to 6 hours of the end of injection, while "delayed abortion" means abortion of (usually) dead fœtuses 36 to 48 hours after the last injection. "Doubtful result" includes cases where the fœtuses are still-born at term, where some of the litter are aborted and some carried to full term, etc.

Control experiments. The mice used in the experiments discussed in the present paper were all 11 days or more post-coitum, when pregnancy is not easily disturbed by manipulation, but since it was previously shown (12) that pregnancy may be disturbed during the early stages by the mere injection of large amounts of fluid, certain control experiments were undertaken. These were as follows: (*a*) 19 injections over 36 hours of an ether extracted and therefore inactivated œstrin preparation, in amounts of 0·5 c.c. per injection, (*b*) injection of 3·0 c.c. of distilled H_2O in one dose, and (*c*) 1·0 c.c. injections of 50 p.c. olive-oil emulsion in

$N/18$ Na acetate, the neutralization salt in the œstrin. Fifteen mice used all had normal litters at term.

III. Effect of posterior pituitary preparations on pregnant mice.

"Pituitrin." Forty-six pregnant mice were injected with "pituitrin" in amounts ranging from 0·01 unit to 2·5 units, the stage of pregnancy varying from 11 to 18 days post-coitum. The results are summarized in Table I. The positive results were mostly straightforward abortions, but

Table I. Effect of "pituitrin" on pregnant mice.

Days pregnant	Units of "pituitrin" 0·01	0·1	0·5	1·0	1·5	2·0	2·5
11	.	.	− (2)	+	−(2)	+(2)	.
12	−	.	− (2)	−(2)	±	+	.
13	.	−	−	+	+	+	+
14	.	.	−	+	−	+	.
15	.	.	−	+	+	+	.
16	.	.	−	(+)	+	+	.
17	−	.	±	+	+	+	.
18	.	−	(+) −	+(2)	+	+	.

(− indicates a negative, + a positive and (+) a doubtful result.)

in the earlier stages a few mummifications and resorptions occurred. The two doubtful results comprised (*a*) still-birth of a litter, and (*b*) abortion of one fœtus and normal gestation of the remainder. These results show that the administration of one or more units of "pituitrin" terminates gestation in the mouse with comparative regularity. It should be noted, however, that the effect consists in the abortion of dead fœtuses 1 to 2 days after injection, and is not therefore any real simulation of a parturition mechanism.

Oxytocin. It was naturally assumed that the results of injection of "pituitrin" were due to its oxytocic activity, but subsequent experiments with the separated oxytocin unexpectedly cast doubt upon this supposition. Eighteen mice varying from 12 to 18 days pregnant were given from 1 to 5 units of oxytocin in one injection, and of these 14 showed no effect whatever, while 2 gave doubtful and 2 positive results (Table II). Following these experiments 5 pregnant mice were given 2·5 to 4 units of oxytocin as a series of four or five injections over 2 to 3 hours. This prolongation of the oxytocic action failed, however, to increase its efficiency: all 5 mice proceeded to full term and normal parturition. Since the largest amount given to these mice (5 units)

TABLE II. Effect of oxytocin on pregnant mice.

Days pregnant	Units of oxytocin 1	2	3	4	5
12	.	.	−	.	−
13	.	.	−	.	−
14	.	.	.	+	+
15	.	.	−	.	−
16	−	.	−	.	− (4)
17	.	.	(+)	.	− (+)
18	.	−	.	.	.

(− indicates a negative, + a positive and (+) a doubtful result.)

represents about twice the normal clinical dose, it must be concluded that oxytocin in physiological amounts will not produce abortion in mice.

Further experiments, in collaboration with Miss M. Hill, are now being carried out on guinea-pigs and rabbits. Preliminary results indicate that 20 to 30 units of oxytocin may be administered to these animals during pregnancy without causing uterine contraction of any intensity. These results, though in keeping with those of Knaus on the isolated organ, are highly discrepant with the effects of "pituitrin" injection, and experiments were, therefore, carried out on the results of injection of the separated pressor principle during pregnancy.

Vasopressin. Eight pregnant mice were injected with vasopressin. Of these, 6 aborted, 1 had a partially still-born litter, while 1 only had a normal litter. Details are given in Table III. These remarkable results

TABLE III. Effect of vasopressin on pregnant mice.

No. of mouse	Days pregnant	Units of vasopressin	Result
PP 313	16	1	Abortion 2 days later
PP 311	17	2	Two alive at term, some still-births
PP 307	14	3	Abortion 3 days later
PP 314	15	3	Abortion 1 day later
PP 292	17	5	Abortion 1 day later
PP 308	15	5	Abortion 3 days later
PP 317	13	5	Normal litter of two
PP 293	16	10	Abortion 1 day later

adequately explain the difference in the effects of pituitrin and of oxytocin. Since abortion occurred 1 to 3 days later, the effect is probably due to some vascular disturbance in the placenta sufficient to cause fœtal death and subsequent evacuation of the uterus.

IV. Effect of œstrin on pregnant mice.

Various workers(3, 13, 14) showed that the administration of the earlier fat-soluble œstrin preparations[1] in adequate amounts led to the interruption of pregnancy. Since, however, the nature of œstrin preparations has radically changed since most of these experiments were carried out, it was deemed advisable to reinvestigate this effect before attempting to analyse any synergistic action between œstrin and oxytocin. In the ovariectomized animal, a given amount of water-soluble œstrin is far more active when administered as a series of injections given over 24 to 48 hours than when given all at once(1, 8). Thus, four injections over 36 hours are about 50 times as efficient as one injection of the same total amount(8). This effect is due to the very rapid absorption of the comparatively pure preparations of the hormone—rapid absorption being clearly undesirable in the case of a substance whose action should be continuous for some 36 hours. Methods of slowing up the absorption of one dose (as by administration in oil) are unsatisfactory, and the only sound method of securing continual availability of the hormone for 36 hours is repeated injection.

In the circumstances, it was anticipated that it would be difficult or impossible to produce by one injection an œstrous stimulus sufficiently prolonged to cause abortion. This expectation was confirmed. In 47 pregnant mice having a single injection of œstrin varying from 1 to 300 units, only one positive result was obtained (Table IV). Thus the largest amount of œstrin which could be given in one dose at the time these

Table IV. Effect of single injection of water-soluble œstrin on pregnant mice.

Units of œstrin	No. of animals	Days pregnant	Results
1	5	12–17	Negative
2	3	14–16	,,
3	1	15	,,
4	2	15–17	,,
6	1	15	,,
8	2	12–15	,,
10	1	13	,,
20	6	11–16	,,
30	6	12–17	,,
40	12	12–17	,,
90	2	12–13	1 litter normal, 1 still-born
200	3	15–17	Negative
300	3	14–17	2 litters normal, 1 litter aborted

[1] "Fat-soluble" and "water-soluble" in this connection are merely relative terms. The earlier and cruder preparations of œstrin, containing much inert fat, had to be emulsified in dilute Na_2CO_3, but the present comparatively pure extracts can be obtained in a true aqueous solution with the aid of a trace of alcohol(1) or dilute alkali(7).

experiments were performed failed to produce regular abortion. Four injections over 36 hours were then tried (total 360 units). The results again were unpromising (Table V). However, the œstrous stimulus

TABLE V. Effect of multiple injections of water-soluble œstrin on pregnant mice.

Duration of injections (hr.)	No. of injections	Interval between injections (hr.)	Total œstrin (units)	No. of mice	Days pregnant at 1st injection	Results			
						Immediate abortion (see p. 465)	Delayed abortion	Doubtful result	No effect
12	13	1	260	4	14–16	1	–	–	3
36	4	12	360	3	11–15	–	–	1	2
36	13	3	260	6	11–14	–	3	–	3
36	19	2	380	24	11–14	1	17	3	3
48	17	3	40	7	12–15	–	–	–	7

provided by injections at intervals of 12 hours is known to be intermittent (8), so injections were given at intervals of 3 and finally of 2 hours for a period of 36 hours. As shown in Table V, 380 units given at the more frequent intervals produced definite abortion in 18 out of 24 pregnant mice. Since only small batches of mice in similar stages of pregnancy could be collected at a time, the labour involved was appreciable, and the effect was therefore investigated of giving 13 hourly injections over 12 hours. 260 units thus given failed to produce regular abortion, but the œstrous stimulus provided was found sufficient to sensitize the uterus to oxytocin.

It is important to emphasize the time relations of œstrin abortion. In the assay of œstrin, it is found that the maximum vaginal reaction to four doses over 36 hours appears at about 48 hours after the last injection: when 19 injections are made over 36 hours the reaction is rather earlier. With two exceptions, the 22 œstrin abortions recorded in Table V all occurred 36 to 48 hours after the last injection ("delayed abortion"), in other words at the time when the maximum effect in the accessory organs is found.

V. EFFECT OF ŒSTRIN AND OXYTOCIN TOGETHER ON PREGNANT MICE.

The experiments on the synergism between œstrin and oxytocin were based on three conclusions which may be drawn from the work recorded above:

(*a*) That oxytocin fails to cause abortion in mice in amounts up to 5 units owing to the insensitivity of the pregnant uterus to this hormone.

(*b*) That abortion caused by œstrin alone occurs 36 to 48 hours after the last injection.

(*c*) That 260 units of œstrin in 13 doses over 12 hours does not cause even delayed abortion regularly.

Thirty mice, varying from 13 to 17 days pregnant were prepared with 260 units of œstrin over 12 hours and, immediately after the last dose of œstrin, were given from 0·25 to 4·0 units of oxytocin. The results, summarized in Table VI, show that with 1·0 or more units of oxytocin

TABLE VI. Effect on pregnant mice of multiple injections of œstrin with a single injection of oxytocin. Œstrin given in 13 injections over 12 hours. Oxytocin given with last œstrin.

Total œstrin (units)	Total oxytocin (units)	No. of mice	Days pregnant	Results: Immediate abortion (see p. 465)	Results: Delayed abortion	Results: Doubtful result	Results: No effect
130	0·25	3	15–16	–	–	1	2
260	0·25	4	14–17	2	–	–	2
260	0·5	4	14–15	1	1	1	1
130	1·0	2	16	–	–	–	2
260	1·0	13	13–17	7	1	3	2
260	2·0	5	15–17	4	–	1	–
260	4·0	4	14–17	4	–	–	–

immediate (1 to 6 hours) abortion followed with fair regularity. Comparing these results with those following the injection of œstrin alone, it is obvious that the uterus which has been initially subjected to the influence of œstrin is capable of response to oxytocin to the extent required to cause evacuation of the fœtuses. This capacity to respond is in marked contrast with the insensitivity of the normal pregnant uterus to oxytocin.

It is still not possible to postulate a really comprehensive theory of the mechanism of parturition, but the fact that the pregnant uterus can be sensitized to oxytocin by œstrin is strong support of the evidence (discussed on p. 464) that parturition is at least assisted by the uterus attaining a sensitivity to oxytocin hitherto absent during pregnancy.

VI. RETURN OF ŒSTRUS AFTER INDUCED ABORTION.

The pregnant mice receiving 260 and 380 units of œstrin in 13 and 19 injections were mated with a normal male at the time of the last injection and observations were kept as follows: (*a*) vaginal smear following injection, (*b*) occurrence of copulation before abortion, (*c*) time

of next œstrus after abortion or parturition, (*d*) fertility of copulation occurring at this first post-abortion œstrus.

The following conclusions were arrived at:

(*a*) Pro-œstrous changes were usually observed in the vaginal smear after injection. Obvious cornification was less regular, but the state of the vagina during pregnancy makes accurate analysis of its cellular contents difficult. Changes were observed in the vaginal smear even in animals which failed to abort subsequently.

(*b*) Six of the 30 mice observed copulated at 12 to 24 hours after the last injection, *i.e.* about midway between the last injection and abortion. This is a shorter reaction time than is found in the ovariectomized mouse (9). All these six copulations occurred in mice which subsequently aborted. A new pregnancy, of course, did not result from copulation at this time.

(*c*) Spontaneous œstrus returned in from 1 to 4 days after abortion, corresponding to 17 to 21 days after the previous copulation. Since the immediate post-parturition copulation in the normal animal is detected 20 days after the original one responsible for the pregnancy, it is clear from these results that the premature delivery of the fœtuses may expedite the post-partum œstrus. Many of the post-abortion copulations resulted in normal pregnancy, which indicates that ovulation also may take place very soon after abortion.

VII. Summary.

1. The old total posterior pituitary extract, "pituitrin," caused abortion or reabsorption in mice in doses of 1 unit or more (Table I). Oxytocin, however, was found to be practically without effect even in amounts as much as 5 units (Table II). This discrepancy is apparently accounted for by the experimental finding that comparatively small amounts of vasopressin will terminate pregnancy (Table III).

2. It was not found possible to cause abortion regularly by a single injection of "water-soluble" œstrin (Table IV), but 380 units given in a series of injections over 36 hours usually produced abortion in 36 to 48 hours (Table V).

3. 260 units given in 13 injections over 12 hours failed to produce regular abortion, but the administration of 1 or more units of oxytocin immediately after the last injection of œstrin was found to cause almost immediate (1 to 6 hours) evacuation of the uterus (Table VI). This reaction time is in marked contrast to that required (36 to 48 hours) for abortion following the injection of adequate amounts of œstrin.

4. This result is in keeping with Bourne and Burn's(2) report that œstrin will sensitize the uterus to the oxytocic principle of the posterior pituitary body.

5. Œstrus recurs at varying intervals after induced abortion, but usually before it would have appeared if pregnancy had proceeded to term.

6. The bearing of these results upon theories of the initiation of parturition is discussed.

The œstrin preparations used were most generously given to me by Mr G. F. Marrian. The supply of urine of pregnancy used as the source of œstrin was made available by the kindness of Prof. F. J. Browne.

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THE VISCO-ELASTIC PROPERTIES OF FROG'S MUSCLES.

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In a perfectly elastic body, to each length should correspond a certain tension, and to each tension a certain length. When a given change in length, or tension, is applied the body should immediately take the corresponding tension or length. Many elastic substances, however, behave differently, *e.g.* rubber. When extended by a sudden increase in tension they show at first an instantaneous extension followed by a slow further extension (5, 6, 7). Under a sudden increase in length the tension shows a large instantaneous increase, followed by a slow decrease; when suddenly released the reverse occurs. The mechanical work absorbed by a sudden extension is greater than that liberated by a sudden shortening (8, 9, 10). This phenomenon called visco-elasticity, or elastic hysteresis (*elastische Nachwirkung*), can be observed in nearly all substances. Crystals alone show no appreciable elastic hysteresis, all other substances show it more or less.

That a muscle in the excited state shows a series of properties corresponding to those of a visco-elastic system has been shown by Gasser and Hill (1). The behaviour under sudden stress, or under sudden extension, of all visco-elastic substances (rubber, gelatin jellies, etc.) suggests a system partly damped, partly undamped.

A precise dynamical model behaving in the same way is, for instance, a spring carrying at some point in its length a disc which can move vertically in a cylindrical vessel without touching the sides (Fig. 1). One end of the spring is anchored to the bottom of the vessel, and a load, or an extension, can be applied vertically to the other. The vessel is filled with viscous fluid. The visco-elastic properties of muscle suggest the same model. Levin and Wyman (2), investigating the tension-length curve of the excited muscle for stretches and releases at different

constant speeds, came to the conclusion that muscle is mechanically considered a system of this type. When we describe the behaviour of a visco-elastic body as being the same as the behaviour of such a model, we only mean that the lengthening after change in tension and the

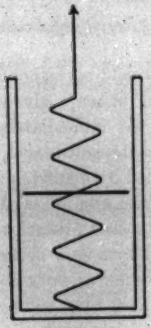

Fig. 1.

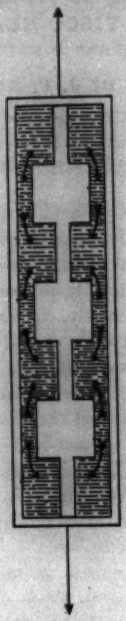

Fig. 2.

Fig. 1. Dynamical model of visco-elastic system.
Fig. 2. Structural model of visco-elastic body.

variation in tension after change in length, follow the same mathematical equations for both.

An explanation of the behaviour of visco-elastic substances might be found:

(1) In certain structural properties (heterogeneous bodies).

(2) In some peculiarities in the liberation of energy (homogeneous bodies).

(1) In a two-phase solid-liquid system, such as a felt of fibrils, or a sponge-like structure, filled with viscous fluid, an increase of deformation with time, *i.e.* creep, will appear when the structure is extended, or shortened. For similar conditions of stress the amount of creep will be greater, the greater the geometrical irregularity of the system (Poole(3)).

An india-rubber model showing such properties is illustrated in Fig. 2. In this model the irregularity in section of the core would cause a flow of liquid in extension or shortening, for the reason that for the same force the extension of the thinner part of the core would be greater than the extension of the thicker part. The irregularity might be due to a haphazard direction of regularly shaped fibrils, or to an irregularity in cross-section of an otherwise regularly directed solid frame.

If the behaviour of a given visco-elastic system corresponds with the behaviour of the dynamical model, and if the structural explanation be adopted, and if the values of the constants determining its behaviour be known, then certain conclusions may be reached about the structure (see Poole(3, 4)).

(*a*) The elasticities of the damped and of the undamped part could be determined, or better a factor, $a_1 l_1$ (elastic coefficient multiplied by length), for the damped part, and a factor, $a_2 l_2$, for the undamped part. The elastic hysteresis is greater the greater the relative value of $a_1 l_1$. We could also determine $K_2 \eta$ (a coefficient of inner friction). An increase in this factor should also enhance the importance of the visco-elastic phenomenon. That a given body shows a considerable elastic hysteresis may mean, either that the damped elasticity is relatively considerable, or that the coefficient of inner friction is great.

(*b*) If we call A_1 the viscous extension, and A_2 the instantaneous extension in response to a sudden increase in stress, the ratio

$$\frac{\text{Viscous extension}}{\text{Total extension}} = \frac{A_1}{A_1 + A_2} = \frac{a_1 l_1}{a_1 l_1 + a_2 l_2}.$$

The relative amount of creep will be smaller the greater the regularity. For the same structure it may be taken as a function of the quantity of mobile liquid contained in it.

(*c*) If we call $K_2 \eta$ the coefficient of inner friction, K_2 will be a factor depending on the nature of the channels through which flow can occur, and η will be the viscosity of the liquid. The term $K_2 \eta$ cannot be split up: the same change will be brought about by a narrowing of the channels as by an increase in the viscosity of the liquid.

(2) Another explanation, however, of elastic hysteresis may be found in a variation in exchange of energy during the performance of work. This kind of hysteresis has been noticed in glass (11). Such an explanation has been put forward lately for the behaviour of muscle by Hartree and Hill (12).

Just in the same way as a relation exists in a gas between pressure (p), volume (v) and temperature (t), a relation may exist in an elastic body between tension (T), length (L), and a variable (I), that we may call an intensity factor, affecting reversibly tension and length; say, for instance, temperature in a metallic wire. Thermodynamic laws similar to those governing the relations between p, v and t will be found between T, L and I.

In a gas doing work, we may have either an isothermic expansion if the gas be in contact with a reservoir of heat at constant temperature, or an adiabatic expansion if there be no provision of heat from outside. In the same way, in an elastic body, we may have an iso-intensity shortening if the elastic body be in contact with an energy reservoir capable of maintaining the intensity factor at a constant level, or an adiabatic shortening if there be no provision of extra energy from outside.

If a gas be in contact with a reservoir of heat at constant temperature, an infinitely rapid expansion will be adiabatic, an infinitely slow one will be isothermal. The pressure-volume relation will vary with the speed of expansion, from the isothermal to the adiabatic curve. In the same way such a gaseous system, suddenly compressed or expanded by a certain force, would at first show an instantaneous adiabatic compression, or expansion, followed by a slow further compression or expansion while the gas came back to its initial temperature. In the case of a gas the ratio: after expansion/total expansion, depends on the ratio: specific heat at constant pressure/specific heat at constant volume, and the factor corresponding to the coefficient of inner friction is the thermal conductivity of the system; in all similar systems, gaseous or elastic, it is the factor regulating the speed with which the isothermic, or iso-intensity, curve is again reached when an instantaneous adiabatic expansion has taken place.

It is easy to see that such a system is mechanically equivalent to the spring model described above, and that the same mathematical equations apply.

This kind of elastic hysteresis, which we may call energetic hysteresis, is very striking in glass, and can easily be demonstrated (Piccard (11)).

A glass tube suddenly bent by a weight shows an instantaneous flexion, followed by a slow further flexion, ending when the initial temperature is again reached; the after-extension proceeds more rapidly in a medium of good thermal conductivity (water) than in a medium of bad conductivity (air).

In every elastic substance this phenomenon should be present. If, for instance, a steel wire under tension be allowed to shorten in a medium at constant temperature, the tension length curve will be isothermal if the shortening be slow; if, however, the shortening be rapid, the wire will heat, the shortening being more or less adiabatic, and the tension for the same length will be smaller.

In other elastic substances another intensity factor might play the part of temperature in the cases just described; all that is necessary is that this intensity factor should markedly and reversibly affect tension and length. In muscle, for instance, a sudden change in some unknown intensity factor certainly occurs on excitation, and strongly affects tension and length. This intensity factor may, for example, be a difference in electrical potential, or an ionic concentration; this factor must then be lowered by shortening; if the shortening be slow, the intensity factor can be maintained at the same level by an energy reservoir somewhere in the muscle; if the shortening be rapid, the intensity factor cannot be maintained at the same level, and the tension for the same length will be smaller.

Any given case of elastic hysteresis might, of course, be due to a combination of those two phenomena, structural and energetic. The visco-elasticity of the excited muscle shows that energetic and structural hysteresis are both important; for: (1) The mechanical potential energy, liberated as actual work during a contraction, is considerably less than the area of the tension-length curve. When a muscle does work during the contraction phase of a tetanic response, the total energy liberated increases. This increase is greater if the shortening be slow, and so the amount of work also is greater. This would be explained if some external reservoir were maintaining the state of tension. On the other hand, no increase of energy appears when the shortening occurs after the stimulus is over, the external reservoir of energy being withdrawn (Hartree and Hill[12].) (2) Gasser and Hill[1], and Hartree and Hill[12], have shown that a muscle behaves as a visco-elastic body, not only during a tetanic contraction, but also when excited by a single shock. In the latter case, however, Hartree and Hill[12] have shown that no variation in total energy can explain the behaviour. The explanation can only be

that there is, in the excited muscle, some structural viscosity, and so we are induced to admit in the excited muscle a two-phase structure geometrically irregular.

We can summarize the position as follows: The excitation of a muscle causes a sudden increase of an intensity factor of some kind, possibly a change in ionic concentration or a difference in electrical potential. This sudden increase causes a change in structure, so that we have in the excited muscle a solid framework showing a certain geometrical irregularity. The formation of this framework might occur by the mechanism suggested by Garner(13). By virtue of this structure the muscle possesses a certain degree of visco-elasticity; by a sudden shortening, moreover, the intensity factor will be decreased, and so the solid framework responsible for the tension will diminish.

Lindhard(14) in his work on "Elasticity of skeletal muscle" criticizes the results of Gasser and Hill(1) as follows: "The most dangerous error especially with regard to the method of Gasser and Hill is that stretching may cause a muscle to contract." The answer was made by Hill(15) that "in the experiments in question the muscle was already subjected to a continuous supermaximal tetanic stimulus applied at both ends, and in such conditions Wyman has recently shown that a stretch considerably diminishes the heat production, so cannot possibly be regarded as further stimulating the muscle." The view of Lindhard is nothing more than the old interpretation of Fick(16) when he suggested an increase of physiological response on stretching the muscle. In Hartree and Hill's interpretation(12) the sudden stretch of an excited muscle increases the unknown intensity factor, a sudden release diminishes it. We may imagine the physical events as follows: the solid frame responsible for tension increases (or decreases) by stretching (or shortening); we are truly in this case increasing or decreasing the physiological response of the muscle (though not the amount of energy it gives out). In a tetanic response this is largely the cause of a muscle's viscosity; if we measure this phenomenon we are not making a "most dangerous error," we are really determining the elastic hysteresis.

Our purpose in the present work has been to obtain some quantitative data of the muscle viscosity. To increase the value of our results we have used in two series of experiments two different kinds of apparatus based on different principles:

(1) The Levin-Wyman ergometer, making for each muscle a series of tension-length curves at different constant speeds.

(2) A muscle extensometer to be described; making for each muscle a series of extension-time curves for sudden change of load.

Theoretically the two methods should give identical results, but by using two different ways of reaching the same end, we more certainly eliminate any systematic error due to the method.

The values of the different constants of muscle hysteresis being known, some conclusions can be reached about the physical state of muscle during excitation.

In this work by E, modulus of elasticity, is meant:

$$\frac{d \text{ force}}{d \text{ extension}} \times \frac{\text{length}}{\text{cross-section}},$$

length and section corresponding to the moment at which this force and extension were observed[1]. As there does not seem to be any variation in volume we may put $l/s = l^2/\text{vol.}$; α the coefficient of elasticity is equal to $1/E$.

Experiments with the Levin-Wyman ergometer.

The Levin-Wyman ergometer records the tension-length curve of a muscle allowed to shorten at any desired speed. The apparatus used in our experiments was essentially the same as the one described in their paper (2). A series of tension-length curves was taken at various speeds. The moving arm of the apparatus opened a contact when set in motion, and closed the same circuit when stopped; the time taken over this known distance was registered by means of an electric signal on a smoked drum, on which at the same time were recorded the vibrations of a tuning fork of 30 D.V. a second. In some experiments the time was checked with a Hipp's chronoscope.

The beginning of excitation, the release of the ergometer and the end of excitation, were adjusted by means of a rheotome, care being taken that the tension should be maximal before the ergometer was released. The excitation was adjusted to outlast the slowest shortening. The preparation used was the frog's double sartorius. The length of the muscle was measured in the body, the limbs being extended; the apparatus was so adjusted that the greatest length of the muscle should be 5/6 of the measured length in the body, and so to be in the rectilinear part of the tension-length curve at zero speed. The amplitude of shortening was $\pm$ 2·3 mm.

A series of experiments was performed at 0°, the muscle being in

[1] When extending a body in which the extension may be great compared with the initial length, it is necessary to take this definition of the modulus. See Villari(1).

oxygenated Ringer's solution on the electrodes, and the vessel containing the electrodes being in a Dewar flask filled with ice. The Ringer's fluid used contained phosphate as recommended by Hill(18). The first excitation was taken when the muscle had already been for 1 hour on the electrodes. To avoid any fatigue we waited 10 min. after each excitation. In each experiment a tension-length curve (speed zero) was made, by measuring the maximum tension at various lengths, for a better determination of the total modulus of elasticity.

The muscle was attached to the isometric lever of the Levin-Wyman ergometer by means of a thin glass rod; this prevents any extraneous elasticity factor from complicating our results. Observations were made alternately at low and at high speed, in order to avoid any systematic error.

The curves were taken on a smoked glass plate. Since the plate was not moved during an experiment, all the curves were superimposed; any fatigue, therefore, could easily be detected by a decrease in tension at the greater length. In all the experiments quoted, no symptoms of fatigue appeared.

The curves so obtained were projected and drawn with large amplification, their areas being measured with the Amsler planimeter and the values obtained reduced to g. cm. of work. In this way a work-speed diagram was constructed for each muscle.

In our experiments we deliberately used shortenings only, because lengthenings very easily injure frog's muscle, as pointed out by Levin and Wyman, and so prevent accurate measurement.

If the muscle behaves as the spring model, we can from the tension-length curves, and the total modulus of elasticity, determine the values of the different constants.

Let E_1 and E_2 denote the respective moduli of elasticity of the visco-elastic, and of the purely elastic part, l_1 and l_2 their respective length, E_t being the total modulus of elasticity, η the coefficient of inner friction, F the tension, $\overline{F}$ the "reversible" tension at the same length, v the speed of shortening, or lengthening, y the extent of shortening or lengthening from the initial muscular length. The form of the equation will then be (Levin and Wyman(2), p. 229)

$$F = \overline{F} \pm \frac{(E_2/l_2)^2 \eta v}{(E_1/l_1 + E_2/l_2)^2} \left[1 - e^{-\frac{E_1/l_1 + E_2/l_2}{\eta v} y}\right].$$

By integration of this equation over an interval Δy we obtain the equation of work given by a shortening or a lengthening of amplitude Δy (Levin and Wyman(2), p. 231),

$$W = \overline{W} \pm \frac{(E_2/l_2)^2 \eta v}{(E_1/l_1 + E_2/l_2)^2}\left[\Delta y - \frac{\eta v}{E_1/l_1 + E_2/l_2}\left(1 - e^{-\frac{E_1/l_1 + E_2/l_2}{\eta v}\Delta y}\right)\right] \quad \ldots\ldots(1),$$

$\overline{W}$ denoting the reversible work during the shortening.

If we solve equation (1), we can determine E_1/l_1, E_2/l_2 and η. Let

$$X = \frac{v}{\Delta y} = \xi x, \qquad \text{where } \xi = \frac{E_1/l_1 + E_2/l_2}{\eta} \qquad \ldots\ldots(2),$$

$$Z = \frac{\overline{W} - W}{\Delta y^2} = \zeta z, \qquad \text{where } \zeta = \frac{(E_2/l_2)^2}{E_1/l_1 + E_2/l_2} \qquad \ldots\ldots(3).$$

Equation (1) then becomes

$$z = x - x^2(1 - e^{-1/x}) \qquad \ldots\ldots(4).$$

The relation between Z and X, deduced from the observed work-speed curve, must be of the same form as equation (4), amplified in the ratios ξ and ζ for the two axes respectively. We may find ξ and ζ by plotting the observed Z/X relation on double logarithmic paper; a standard z/x relation is similarly plotted from equation (4); the amounts of shift parallel to the two axes required to make the second curve coincide with the first give $\log \xi$ and $\log \zeta$. E_t, the total modulus of elasticity, is already known from the "reversible" tension-length relation. We can easily establish the relation

$$E_t = \frac{E_1 E_2/l_1 l_2}{E_1/l_1 + E_2/l_2}.$$

By substituting in equation (2) and (3) and solving, we find

$$E_1/l_1 = E + E^2/\zeta; \quad E_2/l_2 = E + \zeta; \quad \eta = (\zeta + E)^2/\zeta\xi.$$

Alternatively to the use of double logarithmic paper, we may proceed as follows:

$$\frac{Z}{X} = \frac{\zeta}{\xi}\cdot\frac{z}{x} = \frac{\zeta}{\xi}\{1 - x(1 - e^{-1/x})\} = \frac{\zeta}{\xi}\left\{1 - \frac{X}{\xi}(1 - e^{-\xi/X})\right\}.$$

Fig. 3. Levin-Wyman ergometer tension-length curves at various speeds. Amount of shortening 0·275 cm.

The slope of the tangent at the origin of the Z/X curve can easily be shown, by differentiation, to be ζ/ξ. Divide the observed values of Z/X by this quantity and plot against X. The relation $u = 1-(X/\xi)(1-e^{-\xi/X})$ can easily be plotted for various values of ξ, and the abacus so made superimposed upon the previous curve. This gives the value of ξ, when simple multiplication gives ζ.

As an example the calculations are given shortly for the experiment in which the tension-length curves of Fig. 3 were made with the Levin-Wyman ergometer.

Date: 3. iv. 29. Length of muscle: 3·8 cm. Weight: 0·245 g. Volume: 0·230 c.c. The reversible tension-length curve gives E (not reduced to unit length and section) = 121·11.

In this experiment $\Delta y = 0{\cdot}275$ cm. and $\overline{W} = 21{\cdot}31$ g. cm. We obtain for tension-length curves at various speeds:

t (sec.)	W (g. cm.)	$X=\frac{v}{\Delta y}=\frac{1}{t}$	$Z=\frac{\overline{W}-W}{\Delta y^2}$	Z/X
0·352	17·00	2·49	54·25	21·79
0·325	16·43	3·08	64·53	20·96
0·272	15·51	3·68	76·65	20·83
0·204	14·25	4·92	93·31	18·96
0·142	13·32	7·06	105·64	14·97
0·115	12·88	8·69	111·51	12·83
0·105	11·99	9·52	123·26	12·94

Taking X as abscissa and Z as ordinate, we plot the Z/X curve. In this curve the slope of the tangent at the origin is $(dZ/dX)_0 = 27{\cdot}93$. The Z/X values are reduced to the scale of the abacus by dividing by $(dZ/dX)_0$, and plotted against X.

This curve being superimposed on the abacus we choose the value of ξ on the $f(X)$ curve best fitting all experimental values; then calculate the value of ζ, and with the value found for E_t calculate the values of E_1/l_1, E_2/l_2 and η as above.

The values obtained are:

$$E_1/l_1 = 162{\cdot}12; \quad E_2/l_2 = 481{\cdot}41; \quad \eta = 49{\cdot}61.$$

Or reduced to unit length and section:

$$E_1/l_1 = 6{\cdot}60 \times 10^2; \quad E_2/l_2 = 19{\cdot}60 \times 10^2; \quad \eta = 1{\cdot}85 \times 10^3.$$

In Table I are given the values of the different constants in the whole series of experiments, together with R, which is the ratio of viscous extension to total extension, or

$$R = (E_2/l_2)/(E_1/l_1 + E_2/l_2).$$

As will be seen, the most constant value is the value of R, ratio of viscous extension to total extension; compared with the values obtained by Poole(3) for gelatin jellies (0·05 to 0·12 according to temperature) this value seems exceedingly high. The values of E_t, E_1/l_1, E_2/l_2 and η, on the other hand, show a much greater variation; this is probably due to the fact that the reduction to unit section cannot be very accurate, a muscle may contain inactive tissue in amount varying from one muscle

TABLE I.

Date	E_1/l_1 10^9	E_2/l_2 10^9	R	Et 10^9	η 10^9
20. xi. 28	1·75	12·18	0·874	1·52	0·41
22. xi. 28	3·03	12·92	0·810	2·42	0·67
27. xi. 28	2·96	12·08	0·803	2·37	0·79
30. xi. 28	2·99	13·12	0·817	2·50	1·00
4. xii. 29	6·33	24·69	0·796	5·03	1·40
6. xii. 28	5·23	25·09	0·827	4·30	0·89
14. xii. 28	3·91	23·76	0·859	3·34	1·14
5. ii. 29	4·73	12·71	0·729	3·43	0·77
8. ii. 29	3·86	18·94	0·831	3·20	0·73
22. ii. 29	4·13	15·12	0·785	3·24	0·88
4. iii. 29	4·20	15·92	0·791	3·32	1·29
22. iii. 29	4·76	12·25	0·720	3·41	0·86
27. iii. 29	3·98	6·10	0·605	1·17	0·46
3. iv. 29	6·60	19·60	0·748	4·93	1·85
8. iv. 29	1·86	6·94	0·789	2·55	0·74
12. iv. 29	2·21	10·03	0·780	1·80	0·86
18. x. 29	2·51	19·17	0·884	2·14	1·02
21. x. 29	2·11	10·70	0·835	1·72	0·61
28. x. 29	2·42	18·24	0·883	2·07	0·97
8. xi. 29	2·94	13·43	0·820	2·40	1·83
Mean	3·83	15·92	0·799	2·84	1·005
Probable error	±0·0212	±0·821	±0·1327	±0·1538	±0·0620

E_1/l_1 means elastic modulus of damped part.
E_2/l_2 means elastic modulus of undamped part.
E_t means total modulus of elasticity.
R means ratio (viscous extension)/(total extension).

to another, the active cross-section may be a variable fraction of the total cross-section. For the same gelatin Poole(4) admits an error of 10 p.c., from one experiment to another, in the determination of the coefficient of internal friction. We may easily admit that experimental conditions in muscle are more variable than in gelatin.

EXPERIMENTS WITH MUSCLE EXTENSOMETER.

In this second series of experiments we have studied the change of length, as a function of time after a sudden change in tension. Experiments of this kind have very often been made on visco-elastic substances (3, 4, 5, 6, 7). In most cases a very simple device may be used for changing the tension; it may, for instance, be a balance loaded or unloaded at one side, the body to be studied being attached to the other side. The change of length may then be followed by means of a cathetometer.

Since the curves show an instantaneous extension followed by a slow viscous extension, the rapid movement due to the instantaneous extension is deformed owing to the inertia of the rapidly moving mass. This is of little importance when the creep phenomenon lasts for many minutes;

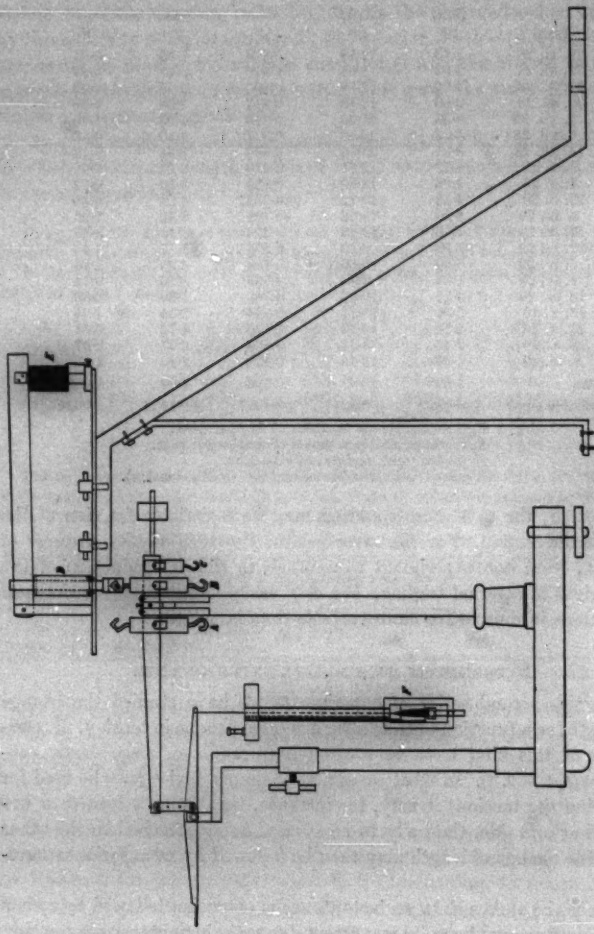

Fig. 4. Muscle extensometer.

a deformation of a fraction of a second is then immaterial. For frog's muscle, on the contrary, the whole curve must be taken in about 1 second, and this cause of error eliminated. The inertia of the moving parts of the system, therefore, had to be reduced and the change in length recorded photographically. To diminish the effect of inertia as much as possible we have reduced the speed of the moving masses in the ratio of 1 : 100, the mass being at the same time increased a hundredfold; for the same speed of shortening of the muscle this diminishes the kinetic energy of the system to one-hundredth. The extensometer used is reproduced in Fig. 4.

The apparatus is essentially composed of two levers, so joined that the total reduction is 100. By changing the weights on *A* or *B* we could pass from one extending force to another. A constant weight placed in *C* maintained after the release any amount of tension desired. The weight on *A* or *B* could be lifted instantaneously by means of a spring *D*, the spring being held in position by an electromagnet *E*.

The moving plate in the photographic recording apparatus opens successively three contacts: the first opening removes a short-circuit and so starts excitation, the second one breaks the circuit of electromagnet *E*, and so the weight in *A* or *B* is lifted, the third one breaks the primary circuit of the stimulating coil and so ends excitation. The muscle preparation is placed in *F* on the electrodes, the vessel containing the electrodes being in a Dewar flask at 0°. The preparation used was the frog's double sartorius as described above. The three contacts of the

Fig. 5. Record obtained with muscle extensometer. Change of load 20·5 to 9·7 g. Time 1/5 sec.

photographic recording apparatus were so adjusted that the unloading of the muscle occurred when the contraction was already maximal. As with the Levin-Wyman apparatus shortenings only were used, a sudden extension very easily injuring frog's muscle. The muscle rested for at least 10 min. after each excitation.

As will be seen in Fig. 5, the curves obtained show a straight vertical part at first and a curved part later; already confirming the comparison of the muscle with the two-spring system.

Now in a body mechanically equivalent to the spring model (Fig. 1) the motion of the disc, after the tension is suddenly altered at the free end, is controlled by three factors: (*a*) the constant force C in the undamped spring; (*b*) the variable force, $K_1 A_v$ in the damped spring, K_1 being a factor proportional to its modulus of elasticity and A_v its extension (referred to as the viscous extension); (*c*) the viscous force $K_2\eta\,(dA/dt)$, where $K_2\eta$ is the coefficient of inner friction and dA/dt the velocity of the disc. As the total extension is equal to $(A_v + A_i)$, A_i being the instantaneous extension: A_i being constant,

$$dAv/dt = dA/dt.$$

Equating the forces on the disc and neglecting its inertia, we have

$$K_1 Av + K_2\eta\,(dA/dt) = C.$$

Adding $K_1 A_i$ to each side and putting $A_v + A_i = A$, this becomes

$$K_1 A + K_2\eta\,(dA/dt) = C + K_1 A_i = \text{a constant} \quad \ldots\ldots(1).$$

Differentiating, this gives

$$\frac{d}{dA}\left(\frac{dA}{dt}\right) = -\frac{K_1}{K_2\eta} \quad \ldots\ldots(2).$$

If the variables be regarded as A and dA/dt, equation (1) shows that the relation between them is linear, its slope being directly proportional to the modulus of elasticity of the damped spring and inversely proportional to the coefficient of inner friction.

If the muscle be really equivalent to the two-spring system, we should find $d\,(dA/dt)/dA$ constant in each curve; as Poole(3) did for gelatin jellies: this we have found, in fact, to be the case.

To obtain the values of the various constants, we may proceed as follows: the value of

$$R = (\text{viscous extension})/(\text{total extension})$$

may be found directly if we know the final extension and the instantaneous extension; this can be done in each experiment by plotting dA/dt as a function of A ("slope-extension curve"). The final extension is given by

the intersection of the slope-extension curve with the extension axis. The exact point where the instantaneous extension ended and the viscous extension began is difficult to determine directly, but easy to interpolate. By integrating equation (1) we obtain

$$\log [(C/K_1) - A_v] = - K_1 t/K_2 \eta + \log C/K_1.$$

The final viscous extension, therefore, is C/K_1; if the final total extension be A_1 and that at any other time A, the equation becomes

$$\log (A_1 - A) = \text{a linear function of the time.}$$

By plotting $\log (A_1 - A)$ against the time, and extrapolating to time zero, we find the value of the instantaneous extension. Knowing A_t and A_1 we can calculate R. Knowing the instantaneous extension, the total viscous extension and the sudden change of tension at the beginning, we can calculate E_1/l_1 and E_2/l_2. The value of $K_2\eta$ can be deduced from the equation (2) above. It is sufficient afterwards to reduce to unit length and section.

As an example, the calculations are given below for the experiment which gave the curve reproduced in Fig. 5.

This experimental curve was projected and drawn magnified on millimetre paper. From this magnified curve were measured twenty values of dA/dt; those values were plotted as a function of A (extension), and so was obtained the slope-extension curve of Fig. 6.

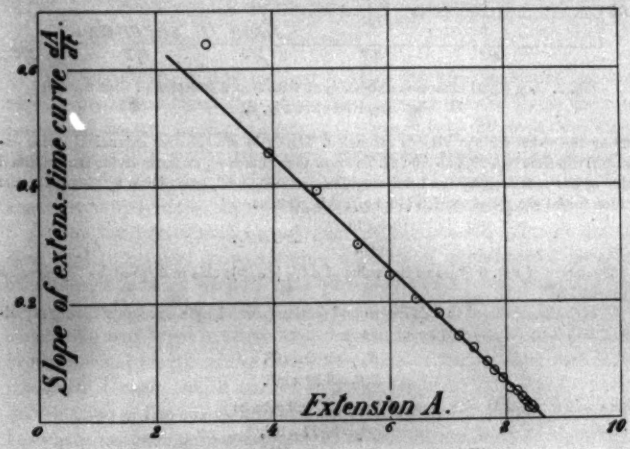

Fig. 6. Slope-extension curve of experiment of Fig. 5.

On this curve an easy extrapolation to slope O gives the final extension (8·65). The instantaneous extension is then found by taking the values of log (total extension-length at any moment) and plotting those values as a function of time. This gives the curve of Fig. 7. Extrapolation to time O gives the logarithm of the viscous extension; this gives

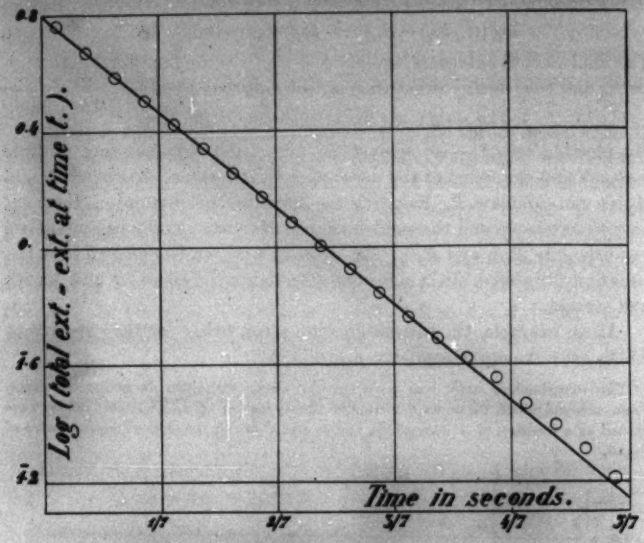

Fig. 7. Log (total extension-extension at time t) as a function of time for the experiment of Fig. 5.

6·29 as the value for the viscous extension. The ratio R=(viscous extension)/(total extension) will thus be 6·29/8·65=0·727. To know the value of K_1 we must divide the amplified value of the extension by the total amplification 6·29/70·07, and divide by this value the sudden initial change of tension (10·8 g.): this gives us

$$\frac{10{\cdot}8 \times 70{\cdot}07}{6{\cdot}29} = 120{\cdot}3 = K_1.$$

The curve of Fig. 6 gives us the value of $dA/d\,(dA/dt)$; this multiplied by K_1 gives us $K_1\eta = 22{\cdot}15$.

These values reduced to unit length and section (mean length of muscle during experiment, 2·82 cm.; volume 0·172 c.c.) give

$$K_2\eta = 1{\cdot}02 \times 10^3,$$
$$E_1/l_1 = 5{\cdot}56 \times 10^3.$$

Those values together with those of R=0·727 will give us

$$E_2/l_2 = 14{\cdot}77 \times 10^3,$$
$$E_1 = 4{\cdot}04 \times 10^3.$$

These values are in accordance with those obtained with the Levin-Wyman ergometer.

In Table II are given the values of the constants for the same muscle from fifteen curves taken in succession. The results are fairly consistent, as consistent at least as those obtained with a gelatin jelly.

TABLE II.

Release 20·5 to 9·7 g. Length of muscle: 3·825 cm.

	L_0/L	$K_{\frac{1}{2}\eta}$ 10^3	R	E_1/l_1 10^3	E_1 10^3
	0·7284	1·13	0·7485	5·26	3·94
	0·7500	1·24	0·7447	5·20	3·87
	0·7275	1·11	0·7261	5·90	4·28
	0·7297	1·02	0·7042	6·12	4·31
	0·7287	1·16	0·7063	5·82	4·11
	0·7303	1·12	0·7116	5·91	4·20
	0·7319	1·06	0·7299	5·74	4·19
	0·7335	1·01	0·7330	5·65	4·14
	0·7321	1·17	0·6882	5·88	4·04
	0·7339	1·24	0·7362	5·07	3·95
	0·7326	1·30	0·7263	5·17	3·76
	0·7334	1·31	0·7399	4·92	3·64
	0·7370	1·02	0·7272	5·56	4·04
	0·7380	0·94	0·7567	5·28	4·00
	0·7324	0·92	0·7310	5·15	3·76
Mean	0·7333	1·12	0·7273	5·53	4·02
Mean error (p.c.)		8·74	1·86	5·65	3·96

L_0/L means ratio (length during experiment)/(normal resting length).
R means ratio (viscous extension)/(total extension).
E_1/l_1 means elastic modulus of damped part.
E_1 means total modulus of elasticity.

One advantage of the method is that the measurements are very rapidly made and, since the values for the same muscle under the same conditions are sufficiently consistent, it is possible to follow the variations of the constants under conditions where the state of the muscle is continuously changing. Another advantage is that the calculations are more rapidly done than with the Levin-Wyman method.

When the experimental conditions are comparable different muscles give similar results; in performing a series of experiments, however, on different muscles we noticed a variation which could not have been detected in the experiments with the Levin-Wyman ergometer. The shortening employed in those experiments, the greater length being 5/6 of the normal length in the body, was done in the straight part of the reversible tension-length curve; at a shorter length we should have a deformation of the curve, and also the tension under quick release might have attained zero, and compressions being impossible, this would give a false result for work at this speed. This prohibited an investigation, with

the Levin-Wyman apparatus, of the influence of initial length. With the extensometer, however, it is easy to do the same experiments at various lengths, variation in tension of 10 g. can be done by passing from 20 to 10 or from 30 to 20 or from 40 to 30; this gives each time a different value for L_0/L (length during experiment)/(normal resting length). We should only compare results taken at the same value of L_0/L. When this value was changed we noticed that R = (viscous extension)/(total extension) is a function of L_0/L. The value of R decreases with a decrease in initial length.

Table III shows the results of an experiment in which the variation of R was investigated as a function of L_0/L. As will be seen the other constants are not affected in the same way.

TABLE III.

Release	L	L_0/L	$\frac{K_{1\eta}}{10^3}$	R	$\frac{E_2/l_2}{10^3}$	$\frac{E_1}{10^3}$
20·5 to 9·7 g.	3·995	0·7326	1·57	0·6994	5·98	4·18
,,	,,	0·7311	1·63	0·7151	6·21	4·44
30·8 to 20·2 g.	,,	0·7682	2·05	0·7396	7·29	5·39
,,	,,	0·7686	1·58	0·8277	6·51	5·39
,,	,,	0·7683	1·85	0·7569	7·25	5·49
,,	,,	0·7764	1·81	0·7834	6·38	5·00
40·8 to 30·1 g.	,,	0·8304	1·53	0·9445	4·93	4·66
,,	,,	0·8289	1·48	0·8967	5·51	4·94
,,	,,	0·8318	1·76	0·8997	5·43	4·88
30·8 to 20·8 g.	,,	0·8007	1·57	0·8783	5·37	4·71
,,	,,	0·8022	1·54	0·8572	5·59	4·79
20·5 to 9·7 g.	,,	0·7676	1·41	0·7686	5·16	3·96
,,	,,	0·7686	1·22	0·7781	5·14	4·00
50·8 to 40·1 g.	,,	0·9412	2·31	0·9623	3·12	3·00

L means normal resting length.
L_0/L means ratio (length during experiment)/(normal resting length).
R means ratio (viscous extension)/(total extension).
E_2/l_2 means elastic modulus of damped part.
E_1 means total modulus of elasticity.

Fig. 8 shows this variation graphically.

In the whole series of experiments the values of R showed the following variation:

0·659 for L_0/L = 0·556 (mean of 4 experiments).

0·7414 for L_0/L = 0·740 (mean of 31 experiments).

0·8953 for L_0/L = 0·8188 (mean of 5 experiments).

0·9623 for L_0/L = 0·9412 (1 experiment).

This change in the value of R might be the result of a change either in the structural properties, or in the energetic properties, of a muscle with length. If it be due to structure, from what has been said before,

we may conclude that the geometrical irregularity increases with lengthening.

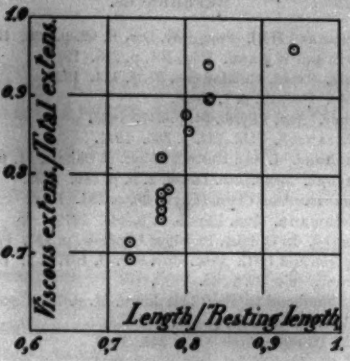

Fig. 8. (Viscous extension)/(total extension) as a function of length.

Summary.

1. By investigating the work done, as a function of speed of shortening, by means of the Levin-Wyman ergometer, the visco-elastic constants of frog's muscle have been determined at 0° and 5/6 of the normal resting length.

2. These results have been confirmed by studying the curve of extension, as a function of time, after a sudden change of load; it has been shown that the ratio (viscous extension)/(total extension) increases with lengthening.

3. There can be no doubt that the muscle behaves as a viscous-elastic system of the damped undamped two-spring type. The nature of this visco-elasticity is discussed; it is to be attributed partly to simple structural viscosity and partly to energetic hysteresis of a type described.

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ACTION OF ADRENALINE AND RELATED SUBSTANCES ON RESPIRATION.

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Oliver and Schäfer(1) first described the depression of respiration which may be produced by injection of adrenaline. Roberts(2) concluded that adrenaline constricted the blood vessels supplying the bulb, and that the resulting acute anæmia produced temporary weakening or paralysis of the respiratory centre. Mellanby and Huggett(3) observed adrenaline apnœa in the decerebrate preparation and after section of the vagi; they also pointed out certain resemblances between adrenaline apnœa and vagal apnœa. More recently, Heymans and Heymans(4) found that the injection of adrenaline (and of certain other hypertensive agents) into the trunk of an animal united only by means of its vagi with the isolated head, arrested respiration in the head; they therefore concluded that the apnœa was reflex in origin. Finally, Heymans and Bouckaert(4a), extending the work of Koch(4b), found that distension of the sinus caroticus in the dog reflexly depressed respiration, and that (in this animal) after section of depressor and sinus nerves adrenaline does not affect breathing, except that a very short respiratory inhibition followed by stimulation may sometimes occur just at the moment of the strong adrenaline blood-pressure rise.

The problem has been re-investigated in the intact cat under chloralose anæsthesia, and in the decerebrate preparation. Respiratory movements were recorded by means of a balloon in the abdomen.

Results.

Effects of cerebral anæmia on respiration.

In view of the theory of Roberts(2) quoted above, the effects of acute cerebral anæmia (produced by obstructing both vertebral and carotid arteries) were studied. In the cat, under chloralose anæsthesia, the breathing soon decreases in depth, and finally ceases without any

Fig. 1. *A*. Cat—chloralose. Vagi intact. Both vertebral arteries have been compressed by a McDowall clamp. Cannula in one carotid artery. At ↑ the other carotid artery was clipped, producing acute anæmia. Rapid failure of respiration. At ↓ carotid clip removed; breathing recovers. Time in 2 sec. *B*. Cat—decerebrate. Vagi intact. At $\overset{\uparrow}{V}$ vertebral arteries compressed. At $\overset{\uparrow}{C}$ carotids clipped. Hyperpnœa and finally respiratory failure. Breathing was restored when blood vessels were released ($C\downarrow$, $\downarrow V$).

Fig. 2. *A*. Cat—chloralose. Vagi cut. Inject 0·2 mg. adrenaline. Typical apnœa. Time in 2 sec. *B*. After denervation of both sinuses. Inject 0·2 mg. adrenaline. No apnœa.

stage of hyperpnœa; soon after the blood supply to the centre is restored respiration is resumed (Fig. 1 *A*). In the decerebrate preparation, however (in which an adequate interval has been allowed to elapse for the volatile anæsthetic to be eliminated), the effects of acute anæmia are quite different. Obstruction of the vertebral arteries alone causes acceleration of respiration and, when the carotids are occluded too, there is further considerable increase in both rate and depth of breathing. Respiration fails after a longer interval (*e.g.* about 40 sec.); recovery occurs when the blood vessels are released (Fig. 1 *B*). It is clear from these observations that the acute apnœa, which is produced quite typically by adrenaline in the decerebrate preparation, must be due to some other cause than acute bulbar anæmia.

Rôle of vagi and of nerves from sinus caroticus.

It is found that adrenaline apnœa can no longer be produced in an animal with both vagi cut, if the carotid sinuses on the two sides are completely denervated (Fig. 2). A definite sinus caroticus is usually visible in the cat, either at the bifurcation of the common carotid artery or on the origin of the internal carotid, or else it may form a spherical swelling from which the minute internal carotid springs. The region of the sinus is surrounded by a plexus of nerves, and the filament from the sinus to the glossopharyngeal nerve (nerve of Hering) can usually be found. To denervate the sinus the internal carotid artery and all the branches of the external carotid artery, except the terminal one (the internal maxillary which contributes to the circle of Willis) are tied; all the nerve filaments surrounding the artery are severed and the vessel is completely freed and lifted clean off the underlying nerves and connective tissue. This procedure does not seriously decrease the blood supply to the bulb. In the considerable number of experiments which have been carried out, adrenaline apnœa has never been observed after section of both vagi and denervation of both sinuses.

If the vagi are intact, denervation of the sinuses alone does not prevent the development of adrenaline apnœa; but if the vagi are then divided the apnœa can no longer be produced. On the contrary, adrenaline may not infrequently under these circumstances stimulate respiration.

These observations suggest that, as in the dog (4, 4a), the apnœa is reflex in origin, and that two afferent paths are available, viz. the vagus and nerve of Hering, but that one or other afferent path is adequate to mediate for the reflex.

Rôle of hypertension in production of apnœa.

It is necessary to decide what is the effective stimulus to the afferent nerve terminals. A wide cannula was inserted into the abdominal aorta, just above the bifurcation, and was connected to a fluid compensator as described by Roberts(2); a large amount of heparine was injected intravenously to ensure free movement of the blood in and out of the compensator. It was found quite consistently that, under these circumstances, adrenaline does not depress the respiration but tends to

Fig. 3. *A*. Cat—chloralose. Vagi intact. Compensator in use. Inject 0·2 mg. adrenaline. Slight rise of blood-pressure only. After initial transient decrease in respiration, breathing becomes deeper. Time in 2 sec. *B*. Same animal. Compensator *not* in use. Inject 0·1 mg. adrenaline. Apnœa and slowing of heart.

stimulate it. Thus, in the experiment illustrated in Fig. 3 *B*, 0·1 mg. adrenaline produced well-marked apnœa and slowing of the heart; the

rapid injection of 0·2 mg. adrenaline when the compensator was in use reduced the amplitude of only two breaths during the period that the compensation was not perfect; this was followed by hyperpnœa; no cardiac slowing occurred (Fig. 3 *A*). Subsequent injections of adrenaline (without the compensator) regularly produced apnœa. These results are the reverse of those described by Roberts(3), but are in agreement with those of Heymans and Heymans(4). It follows that it is the rise of blood-pressure given by adrenaline which reflexly produces the apnœa.

Action of tyramine and ephedrine.

Doses of tyramine which markedly raise the blood-pressure decrease the extent and rate of respiration, but not so markedly as does adrenaline (Fig. 4 *A*). No decrease in respiration occurs when the rise of pressure

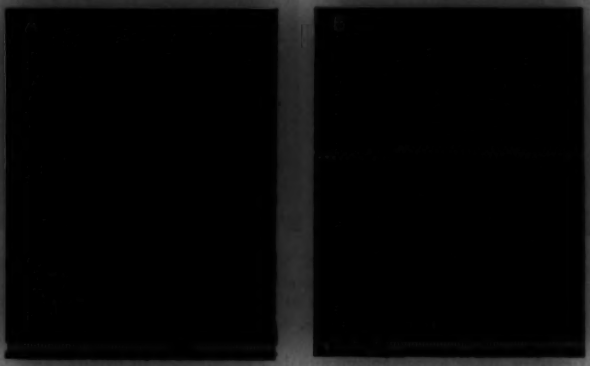

Fig. 4. *A*. Cat—chloralose. Vagi intact. Inject 5 mg. tyramine. Decrease in rate and extent of breathing (the large movements are due to slight convulsive efforts of the body). Time in 2 sec. *B*. Same animal—compensator in use. Inject 10 mg. tyramine. No change in blood-pressure or respiration.

is prevented by means of a compensator (Fig. 4 *B*), or after section of the vagi and denervation of the sinuses. The less-marked respiratory depression produced by tyramine in the intact animal seems to be associated with the fact that the rise of blood-pressure is less rapid in onset and development.

Ephedrine in large doses produces well-marked apnœa (Fig. 5 *A*). A second injection of a large dose lowers the blood-pressure, and may

depress the respiratory centre as indicated by the development of apneustic respiration. In an animal with vagi cut and sinuses denervated a first injection of ephedrine may accelerate respiration (Fig. 5 *B*). The results obtained with tyramine and ephedrine are thus entirely consistent with those obtained with adrenaline.

Discussion.

The experiments described entirely support the contention of Heymans and Heymans(4) that the apnœa produced by adrenaline

Fig. 5. *A*. Cat—chloralose. Vagi intact. Inject 30 mg. ephedrine. Definite apnœa. Breathing recovers though blood-pressure remains at high level. *B*. Another cat. Vagi cut. Sinuses denervated. Inject 30 mg. ephedrine. Sharp initial rise followed by second slower rise of blood-pressure. Increase in rate of breathing.

and certain other hypertensive substances is reflex in origin. One can, therefore, readily understand the finding of Mellanby and Huggett(3) that adrenaline apnœa and vagal apnœa are similar in many respects. The observation by these workers, that adrenaline apnœa is abolished by ergotoxine, is presumably due to the fact that the dose of ergotoxine they employed abolished the pressor action of adrenaline. McDowall's(5) observation that there is a definite parallelism between the apnœa and the inhibition of the heart produced by adrenaline is confirmed by Fig. 3, and is explained by the fact that they are both dependent on afferent impulses from the same sources. Confirmatory evidence has been offered that adrenaline exerts a direct stimulating action on the respiratory centre.

The fact that a slow rise of blood-pressure sets up a less marked reflex than a rapid rise of equal dimensions, suggests that the sensory end-organs are more effectively stimulated by sudden stretch. The breathing frequently returns to normal, though the rise of blood-pressure is maintained; this may indicate that the nerve endings become rapidly "adapted," and are no longer stimulated by the stretched posture of the large arteries.

Conclusions.

1. Adrenaline apnœa is not due to bulbar anæmia; it is dependent (in the cat) on afferent impulses which pass up in the vagus or in the nerve of Hering. It is abolished by section of both vagi if the carotid sinuses are also denervated (confirmatory of Heymans and Bouckaert). The reflex is set up by the rise of blood-pressure.

2. Tyramine and ephedrine produce similar effects on respiration and in the same way.

The expenses of the above research were partially defrayed by a Government Grant through the Royal Society. I am indebted to Prof. R. J. S. McDowall for much advice and assistance.

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PROCEEDINGS
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December 14, 1929.

Improved bearing for the torsion myograph. By J. C. Eccles and C. S. Sherrington.

The usual pattern of the torsion myograph carries the torsed wire on a friction bearing at one end, as in Blix's original form. It is difficult to keep the friction low. Various devices to reduce the friction have proved on trial to get but little success with this form of bearing. Perhaps the most favourable modification of it has been where the steel torsion rod or wire, well burnished, rests on a polished steel surface furnished by a cylindrical small pin below. The torque then causes the torsed wire to roll slightly, and, if the excursion is kept small, the friction, as judged by the freedom of return under calibration tests, is a good deal better than with other forms of friction bearing.

It is, however, of greater advantage to get rid of the friction bearing altogether. For this there is the accurate method used, and we believe introduced, by Hartree and Hill, employing the torsion of a flat and taut steel strip tightly held between two rigid end-clamps. An alternative method is one for which we are indebted to the National Physical Laboratory and the kindness of its Director, Sir Joseph Petavel. In this the bearing end of the torsion wire or rod is, where it rests on its support at the unclamped end, narrowed below to an edge for contact with the supporting surface. This edge is arranged to coincide with the long axis of the torsion rod. The surface of support can have the form of a shallow V-shaped groove for retaining the bearing edge resting in it. The angle of torque when kept small, as it can be under the magnification easily obtained by an optical lever, *e.g.* in the mirror myograph, does not displace the rocking edge of the torsion wire and there is thus no occurrence of side-slip of the bearing edge.

Is so-called secondary excitation of the retina due to stimulation of the rods? By H. E. Roaf (*London Hospital Medical College*).

When a spot of light is viewed slightly eccentrically a blue effect is perceived although the exciting light does not contain any light of short wave-length. This phenomenon was first described by Purkinje.

The "blue arc" effect has been discussed recently by several observers. Troland(5), Amberson(1) and Ellis(2) agree that it is due to nerve

impulses from the stimulated region of the macula causing secondary stimulation of ganglion cells, or unmyelinated nerve fibres, along the path of the nerve fibres by which the impulses are travelling. Troland says that the colour is due to the direction of the electrical current in the nerves. The above explanations seem highly improbable, and it is difficult to see why the colour is always blue no matter what is the wave-length of the exciting light.

Ladd-Franklin(3) suggests that the effect is due to the conducting non-myelinated nerve fibres giving off light which stimulates structures near their path, but she does not produce evidence that conducting fibres do produce blue light.

The phenomenon was frequently observed during experiments on colour vision(4) and ascribed to quite a different cause before it was discovered that the above explanation of it had been given.

It is probable that the effect is due to halation. When one looks at a moderately bright light it is often seen to be surrounded by a haze of the same colour as the spot itself. If, using only one eye, one looks to the outer side of the spot the halo opens out to form a pair of blue arcs or even a blue oval on the outer side of the spot. A similar effect can be produced by forming the image of a spot of light by a large lens and then tilting the lens at an angle. In the first case the image of the spot is surrounded by a symmetrical halo, but when the lens is turned the spot becomes elongated to form a bar and the halo becomes oval. With further turning of the lens the halo spreads out to a faint parabola. If one remembers that the peripheral part of the eye, especially in the dark adapted condition, is more sensitive to light than the central, one can understand why the oval halo becomes visible. Further, if stimulation of the rods produces a blue sensation, then one can understand why the sensation is blue. The latter proposition is to be discussed in a forthcoming communication.

The question arises, however, as to why one does not see blue arcs when looking to the inner side of the spot. Amberson does describe a "blue spike" when looking to the inner side of the spot of light, but this is much more difficult to see. The perception of the "blue arcs" on the outer side is not due to differences between the outer and inner parts of the retina as the sensitivity on both sides of the fovea has been tested. The explanation may be that the optical axis of the eye falls to the inner side of the fovea, hence looking to the inner side of the fovea will make the halo more symmetrical, whilst looking to the outer side will exaggerate the eccentricity.

The "blue arcs" represent a critical condition between the sensitivity of the retina just beyond the *macula lutea* and the change in intensity of the halo with alteration in angle between the beam of light and the optical axis. With too great eccentricity the halo becomes too diffuse to produce more than a faint sensation of light.

On the explanation advanced by the authors mentioned above there is no reason why stimulation of parts other than the outer margin of the yellow spot should not produce secondary excitation from the electrical changes in the nerves or the production of light during the passage of the nerve impulses.

Therefore it is suggested that the "blue arcs" are due to stimulation of end organs outside the macula by the halo which surrounds a light of any wave-length. These end organs are very sensitive to light and produce a blue sensation when stimulated beyond a certain minimal value. It is possible that they are rods.

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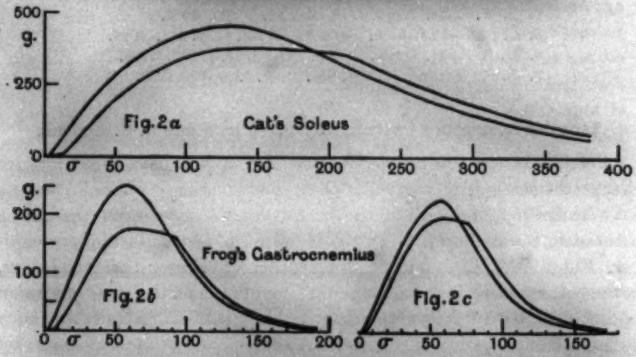
Isometric muscle twitch. By S. Cooper and J. C. Eccles.

Records of the "isometric" twitch of a muscle have shown abrupt changes of tension, in particular they showed sharp "angles" But we have recently found that if the friction at the myograph bearing be reduced to a minimum, the twitch is devoid of any sudden discontinuities. Fig. 1 shows an isometric motor twitch of *extensor digitorum longus*, in the cat, recorded by a "frictionless" myograph (1). Increase of tension begins abruptly $1{\cdot}0\sigma$ after the onset of the electrical response and with no intervening "period of rigidity." A short period of upward concavity is followed by an upward convexity. The relaxation from the curved summit gradually passes into the well-known concave curve of passive relaxation. There is no sign of either "plateau" or "angle." Curves of the "angle" type can be obtained at will by changing the myograph for one of the "friction" kind, all other conditions remaining identical. Similar observations on *soleus*, and median head of *gastrocnemius*, in the cat, and the frog's *gastrocnemius* have left no doubt that the differences between the two types of curves are instrumental.

In Fig. 2 are shown superimposed mechanical records by the two types of myographs. Each pair was obtained from the same muscle in

quick succession and is reduced to the same tension-time co-ordinates. In the phase of contraction the tension registered by the "friction" myograph is always less. This is due to the effect of friction, which increases with the tension owing to the greater pressure at the bearing. After the summit of contraction the return of the "friction" myograph is delayed until the true tension, as revealed by the other myograph, becomes less by an amount equal to the value of friction at that instant.



When twitches are registered with the muscle attached at different points on the lever-arm, it is important to remember that the friction is governed by the pressure at the bearing, and, therefore, is affected but little by alterations in the length of the arm; but, when the muscle pulls through a short lever-arm, it produces by its contraction proportionately less image movement, hence the relative effect of friction is correspondingly increased. Successive myograms, taken from the same muscle under similar conditions, are reduced to the same tension-time co-ordinates in Figs. 2*b* and 2*c*. The differences between the "friction" myogram and the corresponding "frictionless" one are much greater in Fig. 2*b*, taken

with a short lever-arm, than in Fig. 2*c*, where the muscle pulled through a longer arm. The deficiency of tension is greater in Fig. 2*b*. A comparison of the two figures shows that the increase of time to the "angle" in Fig. 2*b* is due solely to a frictional effect. When a muscle pulls through a longer lever-arm, *i.e.*, is allowed more myographic shortening, the "frictionless" myograph shows a slight lengthening of the time of development of the maximum tension.

This raises the question of the significance of the time to the "angle" as a measure of the duration of a muscle twitch. The "angle" corresponds to a point on the relaxation phase of the twitch determined by the length of lever-arm through which the muscle pulls and the amount of friction at the myograph bearing. This latter quantity is dependent on both the muscle tension at that particular instant and the coefficient of friction of the bearing. These complications make the "angle" an unreliable standard. The summit of the "friction" myogram corresponds in time approximately with the summit of the "frictionless" one; using this criterion, it is possible to arrive at the approximate duration of twitches recorded by the "friction" myograph.

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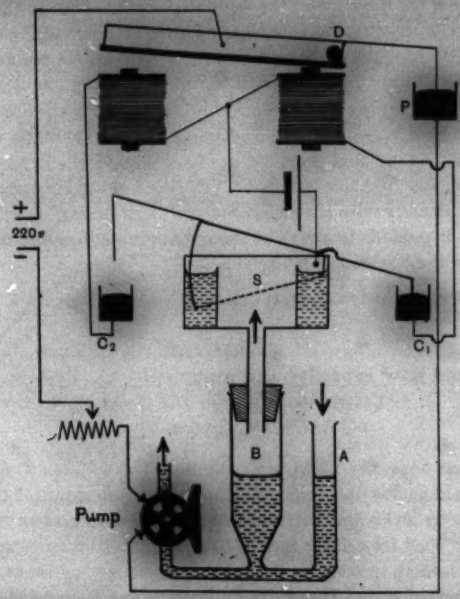
A combined flow recorder and pump control. By L. E. Bayliss and A. R. Fee (*Beit Memorial Research Fellows*). *University College, London.*

It is often necessary when perfusing an organ to allow the blood or perfusion fluid to flow out freely from it and then to return it to a reservoir at a higher level, and for this purpose it is essential that the output of the pump used for raising the fluid should be automatically adjusted to the flow through the organ. While it is possible, of course, to make use of a pump whose piston is not drawn up by a crank, but lifted by the fluid flowing into the cylinder, we have had considerable difficulty in obtaining really reliable valves for a pump of this type, and desired to use the valveless roller pump as described by Bayliss and Müller(1).

The fluid flows from the organ under perfusion into a vessel *A*, about 1 cm. in diameter, which is connected at the bottom to a considerably wider vessel *B* about 4 cm. diameter. This communicates with the small spirometer *S* of 25 c.c. capacity, and the air which is displaced by the fluid rising in *B* lifts the lid of the spirometer until contact C_1 is made. The right-hand coil of the relay is thus energized, the armature, pivoted

at its centre, is pulled down on the right and the mains contact P is made. This starts the pump which draws fluid from the bottom of both A and B, causing the lid of the spirometer to fall (the output of the pump is arranged to be greater than the flow through the organ) until contact C_2 is made, when the left-hand coil of the relay is energized, the mains contact broken and the pump stopped. The relay is stabilized in either the "made" or the "broken" position, in spite of the current through



its coils being only momentary, by a steel ball D which rolls inside a glass tube mounted on the upper side of the armature (a relay of somewhat different pattern, but very suitable for this purpose, is made by Messrs Isenthal).

The time taken for the spirometer lid to rise from contact C_2 to contact C_1 may be taken as an indication of the rate of flow and a signal may be operated by the contacts, or the spirometer arranged to write on a kymograph, as desired; very great accuracy cannot always be obtained when the flow is very rapid, since there is often some surging of the fluid

at the junction of the two vessels *A* and *B* when the pump starts and stops. The sound of the pump stopping and starting may also be used for timing the rate of flow, but an additional error may be introduced owing to its not stopping instantaneously and owing to the slight delay in the operation of the relay.

We have usually set the stroke volume of the spirometer to be 8 to 10 c.c., and the apparatus has worked satisfactorily on flows varying in a few minutes from zero to 200 c.c. per minute. The upper limit to the flow of course is set by the output of the pump; it is advisable to adjust the stroke volume of the spirometer so that it takes at least 3 or 4 seconds to fill.

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The effect of endocrine extracts on the cholesterol content of the suprarenals[1]. By E. NOBLE CHAMBERLAIN.

The work described in this paper was undertaken to determine whether or not overdosage with various ductless gland extracts would modify the functions of the suprarenal glands as shown by some alteration in their cholesterol content. These glands are known to play an important part in cholesterol physiology, and it was thought that their functions might be modified in this respect by feeding with or injections of other endocrine extracts, especially in view of the close inter-relationship of all members of the endocrine system.

Experimental. The extracts chosen were: thyroid administered by mouth in doses of 30 to 70 grains over a period of several days; pituitrin given subcutaneously in single or repeated doses of 1 to 3 c.c.; and insulin given subcutaneously in doses of 30 to 100 units spread over several hours. Buck rabbits were used and were kept on a standard diet of bran, oats and greens. The animals were killed by a blow on the neck and the tissues removed at once for cholesterol estimation. These were carried out by a modification of Grigaut's method in all cases except blood, when the cholesterol was estimated by the methods of Leiboff and Myers and Wardell.

Results. Thyroid feeding resulted in rapid loss of weight of the animal, in some cases as much as a third of the original weight. In spite of this, there was no decrease in the average weight of the suprarenal glands, nor in their cholesterol content. Apart from this fact there was nothing to

[1] Work commenced during the tenure of a Beit Memorial Fellowship and continued under a grant from the Medical Research Council.

suggest any influence of the thyroid extract on the cholesterol content of the suprarenals.

Pituitrin injections, whether single or repeated, produced no changes which can be regarded as outside the limits of physiological variation.

Insulin injections led in almost all cases to a striking fall in the cholesterol content of the suprarenals. The percentage decreased from an average of 7·4 in normal animals to 4·5, whilst the actual amount of cholesterol in milligrams divided by the body weight in kilos. decreased from an average of 16 in normal animals to 9 in insulin injected rabbits.

In some experiments the insulin was allowed to act fully and produce hypoglycæmia, but in others it was thought desirable to prevent this by simultaneous administration of glucose given orally, subcutaneously, or intravenously according to the urgency of the case. In general, it was found that the most marked effects on the suprarenal cholesterol were present when hypoglycæmia was most marked, but the presence of normal or high blood sugars did not entirely prevent the effects on the suprarenal cholesterol due to the insulin.

Other tissues such as blood, liver and spleen were also examined in most experiments for their cholesterol, but the results showed no material alteration from the control figures.

Lactose excretion in the puerperium, and isolation of sugars from urine and blood. By L. B. WINTER. *Physiology Department, St Thomas's Hospital.* (*Preliminary communication.*)

During the puerperium roughly 50 p.c. of women excrete considerable amounts of reducing sugar in the urine. Clinically there is no difference in type, *e.g.* age, parity, quantity of milk secretion, between those that excrete sugar and those that do not. It is stated that lactose is excreted also during the last weeks of pregnancy. To identify the sugar repeated attempts were made to obtain pure specimens of lactose phenylosazone from specimens of urine taken on the fourth day after delivery, when the sugar excretion is at its height. Prepared direct, or after partial separation of the sugar by such means as alcohol extraction, no characteristic lactosazone was obtained. The water-soluble osazones were always different from that of lactose in crystalline form; the melting point varied between 150 and 200° C., and the substance was lævo-rotatory or inactive in spite of repeated crystallization. Lactose phenylosazone was originally described by Neuberg as optically inactive, but I find that specimens show $[\alpha]_{5461}$ = ca. + 35° in pyridine-alcohol.

Hofmeister isolated lactose from the urine of lactating women, but for the present work a method was sought which would be applicable to small quantities of material. Precipitation with basic lead acetate and final purification as the barium compound is successful. 1 g. of lactose added to 200 c.c. of normal urine has been recovered with a yield of 68 p.c. of crystalline sugar melting at 200–205° and showing $[\alpha]_{5461} = +90$, sinking to 60°. Lactose can be readily isolated from the urine of lactating women, and the yield compared with the total sugar content given by the copper reducing power. It is found that:

1. In many cases the yield of crystalline lactose agrees well with the figure found by titration, assuming the same loss in extraction to occur as in the control experiments.

2. The lactose isolated gives an osazone melting at 198–200°, and showing $[\alpha]_{5461} =$ ca. $+35$.

3. No experiment has yet shown that lactose is excreted before parturition.

The lactose excretion is often so great as to suggest that as much lactose is passing into the urine as is being secreted into the milk. During the height of the lactose excretion, the milk has been drawn from the breasts at frequent intervals; no significant change in the lactose content of the urine or milk took place.

To determine whether added lactose could be separated from blood, 0·057 g. lactose was added to 61 c.c. of blood from a cat. 0·021 g. was recovered with melting point 200–205°, and showing $[\alpha]_{5461} = +91$. A further small quantity was removed, and the remaining syrup allowed to crystallize. The crystals showed $[\alpha]_{5461} = +96{\cdot}7$ falling to $+50$ after 18 hours. The solution was then heated with phenyl-hydrazine and acetic acid, and typical glucosazone crystals separated from the hot solution.

The effect of carbon dioxide on certain reflex activities.

By R. J. S. McDowall, *King's College, London.* (*Preliminary communication.*)

If a decerebrate animal is overventilated, the characteristic rigidity disappears and the muscles become quite placid, but the rigidity returns if carbon dioxide is caused to accumulate, due precaution being taken to prevent oxygen want. There appears, however, to be an optimum concentration of carbon dioxide.

On the circulation the effect of sensory stimulation and the capacity of an animal to recover its blood-pressure if placed in the vertical feet-down position are much reduced by overventilation, whether or not the overventilation has caused any alteration of blood-pressure.

These results suggest that if the respiratory centres are considered as the upper ends of reflex arcs, then the well-known stimulating effects of carbon dioxide on these centres are not specific, but are common to many reflex arcs (but not all).

PROCEEDINGS

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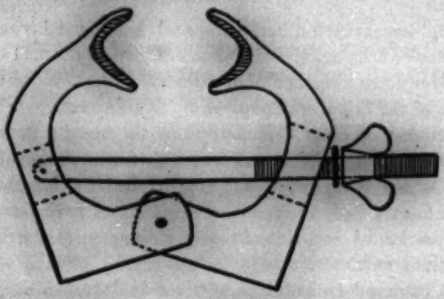
January 18, 1930.

A vertebral clamp. By R. J. S. McDowall.

A clamp has been devised by means of which the vertebral arteries at their entrance to the atlas can be occluded without removal of the skin. It is made of $\frac{1}{4}''$ brass, tapered to $\frac{1}{8}''$ at the jaws, which are wedge-shaped as the shading indicates.



The clamp, together with carotid occlusion, provides a convenient method of testing the activity of the vasomotor and other centres which are stimulated by sudden anæmia, and also an easy way of demonstrating how the reactions of the intact or decerebrate animal differ from those of the spinal animal. The reactions of the spinal animal are obtained in a few minutes on application of the clamp and clipping of the carotids.

A localized lactic acidosis in the brains of pigeons suffering from B_1 deficiency. By H. W. Kinnersley and R. A. Peters.

(*From the Department of Biochemistry, Oxford.*)

The abnormal amounts of lactic acid found in the brains of vitamin B_1 deficient pigeons are now found to be unevenly distributed. The increase is especially marked in the lower parts of the brain. Cerebrum and cerebellum show the same contents of lactic acid. Under our conditions, that is to say with brains fixed within 6 seconds of death by the use of liquid air, the content of lactic acid in the normal brain is about 56 mg.

per 100 g. tissue. Immediately before symptoms appear, lactic acid in the lower parts of the brain tends to approach 70–80 mg. with a normal value for cerebellum and cerebrum. When symptoms appear, the lactic acid rises in all parts of the brain, but the increase is most marked in the lower parts, which may approach 100–120 mg. per 100 g. The results suggest that the symptoms are due to an increase in lactic acid in the lower parts of the brain, and that this is due in turn to a lack of vitamin B_1 in the tissue.

"Amytal" and the inhibitory action of the vagus on the heart.

By R. C. Garry.

"Amytal," isoamyl ethyl barbituric acid, has found favour in recent times as a non-volatile anæsthetic for laboratory animals. Details of its action, effective dosage, method of preparation and of administration and a full review of the literature are given by Mulinos(1).

In the course of class demonstrations on blood-pressure in cats anæsthetized by "Amytal," it was found that stimulation of the peripheral ends of the right or left cut vagus failed to give the expected result on the heart.

A short series of experiments was subsequently carried out to investigate the point.

Fourteen cats and two rabbits were used. In ten a marked vagus action on the heart and on the blood-pressure was obtained under ether anæsthesia or in a decapitate preparation. "Amytal" was then administered intraperitoneally or intravenously and the ether anæsthesia, if present, was withdrawn after a suitable interval.

In all cases the action of the vagus was either completely absent or extremely slight with a strength and duration of stimulus capable of giving a well-marked action before the administration of "Amytal."

In six animals "Amytal" was given at the outset, and in these cases, too, the vagus action on the heart was very slight or absent.

In three cases a record was taken from the auricles and from the ventricles with the same result.

The effective anæsthetic dose is given as 50 mg. per kilo body weight by the makers, but Mulinos gives it as 100 mg. subcutaneously and 70 mg. intraperitoneally. These latter limits were not overstepped in the present series.

Given intravenously even less than 50 mg. per kilo partially or totally obliterated the action of the vagus.

In three cases the preparation was kept alive for several hours after the exhibition of "Amytal." In those cases there was sign of return of the normal vagus activity. Further administration of "Amytal" again annulled the vagus action.

SUMMARY.

"Amytal," in the usual anæsthetic dosage, both in cats and in rabbits, greatly depresses or completely obliterates the normal inhibitory action on the heart obtained by stimulating the peripheral end of either vagus nerve.

1. Mulinos, J. Pharmacol. Exper. Thera. 34. p. 425. 1928.

Sinus caroticus and respiratory reflexes. Cerebral blood flow and respiration. Adrenaline apnœa. By C. HEYMANS and JEAN J. BOUCKAERT. *Pharmacological Laboratory, University of Ghent.*
(*Preliminary paper.*)

In studying the relations between circulation and respiration by the method of the isolated and perfused head, we (J. F. and C. Heymans) have demonstrated in previous publications that a diminution or an increase of blood-pressure, in the cardio-aortic area only, produces respectively a reflex (vagus-depressor) stimulation or inhibition of the respiratory centre. It has been known that occlusion of the common carotid arteries stimulates and that an increase of blood-pressure or blood flow in the carotid cephalic circulation inhibits the activity of the respiratory centre (Cooper, Gesell, C. F. Schmidt, etc.). Tractions on the cephalic end of the common carotid (Sollmann and Brown), stimulations of the sinus caroticus or changes of pressure in the sinus caroticus produce respiratory reflexes (H. E. Hering, Danielopolu, Moisejeff, C. Heymans, E. Koch).

The two isolated sinus caroticus of dogs, anæsthetized with chloralose or urethane, are perfused with the Dale-Schuster pump, the innervation of the two sinus remaining intact; the thoracic respiratory movements, the sinus perfusion pressure, and the femoral arterial blood-pressure of the dogs are registered. With this experimental technique one observes that an increase of the perfusion pressure in the isolated sinus caroticus above the level of 50 mm. Hg produces a reflex slowing of the respiratory rate and a decrease of respiratory amplitude, a high sinus pressure to 200–300 mm. Hg producing a reflex apnœa. On the other hand a lowering of the sinus perfusing pressure produces a reflex

stimulation of the respiration, a reflex hyperpnœa at a low sinus pressure of 60–40 mm. Hg. This reflex stimulation of the respiratory centre by a low sinus pressure may transform a central humoral apnœa (hyperventilation) into a hyperpnœa.

In a dog all the efferent arteries of the sinus caroticus (internal and external carotids, occipital and laryngeal arteries) except the lingual arteries are ligatured, the vagus-depressor nerves are cut, respiration and femoral blood-pressure are registered. A lowering of pressure in the sinus, by clamping the common carotids, produces a reflex hyperpnœa; when the clamps are removed respiratory inhibition results, although there can be no appreciable changes in the cerebral blood flow. The decrease and increase of the cerebral blood flow, on the contrary, by clamping and opening the vertebral arteries do not influence the respiration.

The occlusion of the common carotid arteries or the intravenous injection of adrenaline in dogs, after section of the depressor and sinus nerves, does not produce any further stimulation, reduction or cessation of respiration; sometimes a very short respiratory inhibition, followed by respiratory stimulation, may occur just at the moment of the strong adrenaline blood-pressure rise; this slight inhibition of respiration also occurs after intravenous injection of adrenaline on dogs with carotid and vertebral arteries clamped and depressor and sinus nerves cut.

We have demonstrated in previous publications that an asphyxia or a hyperventilation of the heart-lung preparation, only connected with the isolated head by the vagus-depressor nerves, produces respectively a reflex stimulation or inhibition of the respiratory centre. The two isolated sinus caroticus of dogs are perfused with a Ringer solution of *p*H 7·2, the respiration and femoral blood-pressure are registered. When the normal Ringer solution is changed to a Ringer solution of *p*H 6·4, this increase of hydrogen ion concentration, in the sinus caroticus only, produces a reflex stimulation of the respiratory centre (hyperpnœa), an increase of the general blood-pressure and an acceleration of the heart. Changing the Ringer of *p*H 7·2 or 6·4 to a solution of *p*H 8·3, one observes a reflex inhibition of the respiratory centre (apnœa), a diminution of the general blood-pressure and a slowing of the heart.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

February 8, 1930.

On the rate of oxidation of adrenaline and the assumed sensitization of intestinal muscle by blood, serum and tissue cells. By MARION O. P. WILTSHIRE.

Experiments which were carried out on the intestinal musculature of the rabbit, pieces of duodenum, jejunum and ileum being used, have been devised for the solution of the question whether the effect of serum, liver, brain, lung, voluntary and involuntary muscle is due to the sensitization of intestinal muscle towards minute amounts of adrenaline—a view suggested by O'Connor(1), and held by others(2).

In order to ascertain whether actual sensitization occurs, it was necessary to determine the rate at which adrenaline is destroyed under the conditions of the experiments, *i.e.* $T = 37°–38°$ C., vigorous stirring of the adrenaline solution, concentrations of $1 : 2 \times 10^6$ to $1 : 30 \times 10^6$ adrenaline, aeration with oxygen, and constant *p*H of 7·4, deviations from which are not detectable by colorimetric determination.

It is found that the rate of oxidation can be measured with a fair degree of accuracy. The apparatus used consists of a glass disc which carries a tube of a capacity of 30 c.c., height 12 cm. and diameter 2 cm. In the tube is suspended a piece of contracting jejunum in Locke-Ringer solution, and the contractions are recorded on a slow-moving drum. The tube can be raised so that the piece of contracting gut can be exposed to the 450 c.c. of adrenaline solution of known strength (which is slowly undergoing spontaneous oxidation) at definite times after the addition of adrenaline. The development of varying shades of a pink tint proved to be no certain guide to the rate of oxidation.

The oxidation products of adrenaline themselves exert no obvious effect on the gut, and the point at which all the adrenaline has disappeared can be exactly determined.

The rate of oxidation in each experiment is therefore able to be ascertained for a temperature of 37°–38° C. under the conditions already mentioned for a given piece of gut.

Estimated in this way the rates of oxidation determined for a large number of experiments are as follows:

Strength of adrenaline	Seconds
1 in 30×10^6	18
1 in 20×10^6	22
1 in 10×10^6	30
1 in 5×10^6	50–55
1 in 3×10^6	70
1 in 2×10^6	270

It is found that a remarkable persistence of the effect of adrenaline in concentrations of $1 : 5 \times 10^6$ or less, can be demonstrated when various tissues are added to the solution.

The effect of the following tissues was ascertained: liver, kidney, brain, blood, striated and unstriated muscle. The tissues alone either had no effect at all or only a slight one. When the adrenaline is added simultaneously with the tissues, the adrenaline effect can be obtained 30 minutes later, and probably much later still.

If the tissue is added when presumably all the adrenaline has been oxidized, there is no prolongation of the time during which the adrenaline is effective. But there is a short interval of time in the early stages of the oxidation of adrenaline during which the tissue may be added and the adrenaline effect persist as in the experiments where the tissue and adrenaline are added simultaneously.

Experiments so far have not shown that the tissues bring about a sensitization of the muscle. It is possible that this may occur, or, on the other hand, some interaction between adrenaline and the tissues, which delays the oxidation of the adrenaline, may be the explanation of the persistent adrenaline effect.

1. O'Connor, Archiv f. exp. Path. u. Pharmak. 67. p. 195. 1912.

2. Abelous et Soula, Compt. Rend. Soc. Biol. 96. p. 749. 1922; Schlossmann, Archiv f. exp. Path. u. Pharmak. 121. p. 160. 1927; Abderhalden u. Gellhorn, Pflueger's Archiv, 199. pp. 320 u. 437. 1923; ibid. 203. p. 42. 1924.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
March 15, 1930.

Interaction between distant areas in the human eye. By RAGNAR GRANIT (*Helsingfors and Johnson Foundation, Pennsylvania*).

In order to obtain information as to whether there is any interaction between distant areas in the human eye, the fusion frequency of four circular test patches, separated by an unstimulated area, has been measured for each of the patches in turn and for all of them together. The four stimuli, each of a diameter of 1° of visual angle, have been placed symmetrically with their outer edges on an imaginary circle, the diameter of which has been either 3° (Arrangement I) or 4° (Arrangement II) of visual angle. The test patches have been illuminated at two different intensities, the one 100 times lower than the other. The fixation point has been either in the centre between the stimuli or 10° out towards the periphery.

With central fixation and the higher intensity there is very slight interaction between the stimuli in Arrangement I, as shown by the fact that the fusion frequency of all the patches illuminated together is only about ¼ of a revol. per sec. higher than the corresponding value for each of them viewed in turn. With the weaker intensity there is no interaction at all.

With peripheral fixation under similar conditions there is an increase in the fusion frequency of about 2·5 revol. per sec. for all the patches together over the value obtained with the single stimuli. The interaction is definite still with Arrangement II where the patches are farther apart, the fusion frequency for all of them together being about 1·6 revol. per sec. higher than the average for the singles. Even if the intensity of the stimuli is diminished 100 times there are still definite signs of interaction left.

The results prove the existence of physiological irradiation in the human eye and thereby give a definite solution of an old problem of vision. They are also in general agreement with recent work on interaction in motor and sensory neurones.

The difference between central and peripheral vision with regard to interaction is correlated with corresponding structural differences in the two parts of the retina.

Effects of anterior pituitary preparations on the anœstrous ferret. By MARGARET HILL and A. S. PARKES.

The ferret is peculiar among laboratory animals in having a long period of anœstrus, lasting from August to March. The quiescence of the gonads during this period affords excellent opportunity for investigating the stimulating properties of preparations of the anterior lobe of the pituitary body. The ultimate aim of the experiments here described was to cause the animals to breed during their anœstrous period.

Various preparations of anterior lobe were used, but many of the experiments were carried out with preparations from urine of pregnancy, which has also been shown to contain the active substance. Tissue preparations were made (*a*) by Teel's *N*/10 NaOH extraction method, and (*b*) by acid extraction as used by Bellerby. Urine preparations were made by alcohol fractionation of the alcohol insoluble fraction of the concentrated urine.

Six males and twenty-five females were injected daily for varying periods, and then one gonad was usually removed. The remaining gonad was used to determine the changes taking place after the end of injection, or, in certain animals, injection was continued till the animal was killed.

The following conclusions were arrived at:

(*a*) Ovulation, as shown by the presence of ruptured follicles and tubal ova, can be induced in eight to twelve days. Some of the extracts, however, resulted in the production of large cystic follicles and failed to cause ovulation. The weight of the anœstrous ovary may be increased as much as ten times.

(*b*) These ovarian changes are associated with the development of œstrous changes in the uterus and vagina, and with the vulval swelling typical of œstrus. Complete œstrus can therefore be induced in the anœstrous female.

(*c*) Follicles induced to ovulate in the anœstrous period failed to form corpora lutea unless injection was continued. In either case the œstrous changes in the accessory organs disappeared after ovulation.

(*d*) The injected males responded far less readily than the females, and failed to show either spermatogenesis or appreciable increase in the size of the testis. They were, however, willing to copulate, and it is probable therefore that functional changes were induced in the interstitial tissue. Owing, however, to the lack of seminal vesicles and prostate in the ferret, it was difficult to obtain conclusive evidence on this point.

(*c*) Copulation took place between the treated males and treated females, but was, of course, infertile owing to the absence of spermatozoa.

Ketonuria in rats on a fat diet (*a*) after injections of pituitary (anterior lobe) extract, (*b*) during pregnancy. By J. H. BURN and H. W. LANG.

(*a*) Injections (under the skin) of pituitary (anterior lobe) extract cause a rise in the excretion of acetone bodies in the urine of rats which have been eating a diet of butter for some days(1, 2). The rise may be very great, up to 50 mg. total acetone per rat, but is confined to the 24 hours following the injection. The amount required to produce a rise varies considerably from one rat to another. The extracts have been prepared by extracting the minced gland with *N*/10 sodium hydroxide, and by subsequent neutralization and centrifugation, when the supernatant fluid has been used. A single injection of 0·5 c.c. of a 25 p.c. extract has been found sufficient to cause a large rise.

(*b*) Adult female rats, after mating, were fed with butter only from the 14th day of pregnancy when red blood corpuscles were seen in the vaginal smear. For the first four or five days the acetone bodies in the urine remained small in amount. For the next two days (5th and 6th) a sudden rise in their amount was observed, followed by a fall on the 7th day, which was the day before the litter was born.

A typical result is as follows:

15th	day of	pregnancy	"Total Acetone"		1·0 mg.
16th	,,	,,	,,	,,	1·0 ,,
17th	,,	,,	,,	,,	4·0 ,,
18th	,,	,,	,,	,,	4·0 ,,
19th	,,	,,	,,	,,	15·0 ,,
20th	,,	,,	,,	,,	55·0 ,,
21st	,,	,,	,,	,,	50·0 ,,
22nd	,,	,,	,,	,,	15·0 ,,
23rd	,,	,, (litter born)	,,	,,	9·0 ,,
1st	day post	partum	,,	,,	6·0 ,,
2nd	,,	,,	,,	,,	3·0 ,,

The changes have been observed in ten rats, and only in one was the pre-parturition rise not observed.

1. Burn and Ling. This Journ. 65. p. 191. 1928.
2. Burn and Ling. Quart. J. Pharm. Pharmacol. 2. p. 1. 1929.

Decerebration clamp. By FREDERICK R. MILLER.

The decerebration clamp illustrated in Fig. 1 was designed to replace the thumb and forefinger in making pressure over the vertebral arteries both during and following decerebration, performed by the method of Sherrington(1). After preliminary tracheotomy and ligation of the carotids under deep anæsthesia the head of the cat is secured in a head-holder (made by Messrs C. F. Palmer, Ltd.) and the decerebration clamp is applied from below the neck of the animal; for this purpose the rod *S.R.* is adjusted and held in a swivel-clamp, this latter being mounted

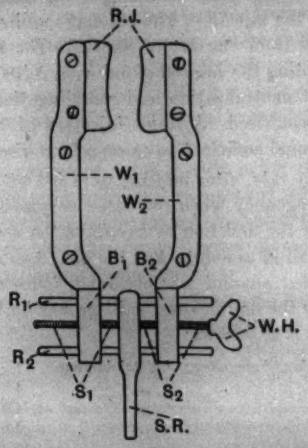


Fig. 1. Decerebration clamp. One-third natural size.

on one end of a brass rod, the other end of which is secured in a Palmer "semi-universal joint," itself fastened low down on the upright rod, which bears the head-holder; the semi-universal joints used to support the head-holder and the decerebration clamp must be of the same style and size. By suitable adjustments of the supporting clamps the jaws *R.J.* are brought accurately to bear over the vertebral arteries just behind the wings of the atlas; each jaw is made of a wedge of soft, red rubber, its thin edge next to the corresponding vertebral artery. The jaws are held in the two wings W_1, W_2, each composed of a front and a back lamina, the two laminæ of each being screwed together so as to grip a

third continuous with each of the blocks B_1, B_2. To ensure equal pressure on both vertebrals it is essential that the rod *S.R.* lie in the same vertical plane as the midline of the skull.

Pressure by the jaws over the vertebrals is applied and regulated by turning the winged head *W.H.*, which operates the reciprocal screws S_1, S_2; the blocks B_1, B_2 slide on the rods R_1, R_2. For effective control of bleeding the neck must be held quite firmly by the jaws; the degree of pressure may be judged by grasping and moving very slightly the neck below the clamp; it is often wise to apply pressure until the respiration shows slight weakening, the anæsthesia having been kept at an even pace. After removal of the cerebrum the pressure may be quickly adjusted so that bleeding is minimal, and this may be checked by packing the base of the skull tightly with cotton wool.

After decerebration the clamp should be left on the vertebrals for about 20 minutes, an interval which usually suffices for complete hæmostasis. The use of the clamp releases one hand of the assistant and affords relief from the unpleasant finger cramp, which often ensues on prolonged holding of the vertebrals. The clamp may further be used for controlling bleeding in cerebellar and other brain operations. The instrument, which is constructed of aluminium and brass, was demonstrated at the Thirteenth International Physiological Congress in August, 1929. The clamp may be obtained from Messrs C. F. Palmer (London), Ltd.

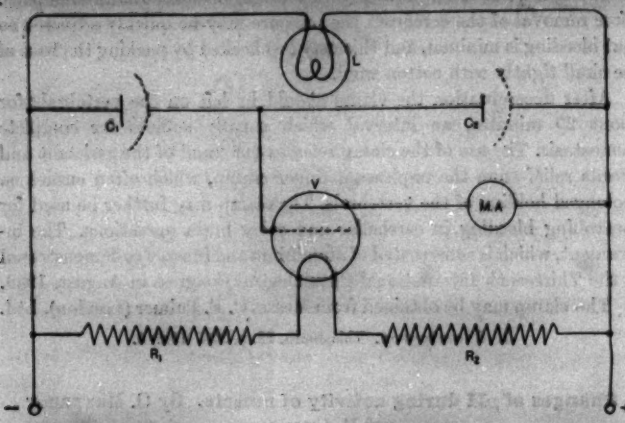
1. Sherrington. This Journ. 22. p. 319. 1897–8.

Changes of *p*H during activity of muscle. By O. Meyerhof and F. Lipmann.

The change of *p*H in frog's sartorii was measured by determining the CO_2 uptake or liberation by means of manometrical methods in an atmosphere of N_2 to which CO_2 of various partial pressures had been added. It was shown that in a prolonged series of twitches the muscle at first becomes more alkaline and more acid only in an advanced stage of fatigue. The change of reaction towards alkalinity is the greater the higher the concentration of CO_2 in the gas mixture. The explanation of the phenomenon is found in the fact that the breakdown of creatine phosphoric acid, which in the first stages of fatigue is large compared with the simultaneous formation of lactic acid, increases the amount of basic equivalents. The change of *p*H can be calculated from the amount of phosphagen split and lactic acid formed and agrees well with the change observed.

A photo-electric hæmoglobinometer. By C. GORDON-WILSON, W. A. M. SMART and F. CAMPBELL SMITH (*London Hospital*).

Continuing the work published in the *Proceedings of the Physiological Society* (1928)(1), the present writers have devised a combination of two photo-electric cells for use as a hæmoglobinometer or colorimeter. The circuit employed is shown in the figure. This circuit has the following advantages. (1) It is a null method, making use of a movable wedge containing the solution of unknown strength. (2) It is operated directly



C_1, C_2 } Potassium light-cells. Osram K.M.V. 6.
V. Osram P. 625.
L 240 volt, 60 watt lamp.
M.A. Milliammeter. 0–30 milliamps.
R_1, R_2 } 600 ohms each.
Mains. 240 volts, D.C.

from the main electric supply, in this case 240 volts D.C. should the potential of the main supply vary by more than two or three per cent., the valve filament may be heated by a 2-volt accumulator. (3) Valve amplification is employed, and a milliammeter is sufficiently sensitive for determining the null point.

In order to attain maximum sensitivity it is necessary to isolate, as far as possible, that part of the spectrum which is to be absorbed. In the case of hæmoglobin a suitable green filter is placed before each light cell. To make a measurement, two identical solutions of hæmoglobin are placed one in each of two absorption cells one centimetre thick. The light

is now moved until the reading of the milliammeter shows that the grid potential will give optimal sensitivity. One cell is then removed, and a glass wedge (or other means of varying the thickness) substituted; this is now adjusted until the milliammeter reading returns to its previous value. The position of the wedge is read on a scale which gives the average thickness of the transmitting portion. This thickness is inversely proportional to the concentration of the standard.

By using suitable filters the method can be applied to solutions of other colours.

The writers wish to thank the Staff of the General Electric Company Research Laboratories for their kind advice. One of us (F.C.S.) was working under the auspices of the Freedom Research Fund of the London Hospital.

1, Campbell Smith and Gordon Wilson. Proc. Phys. Soc. xvi. Nov. 1928.

Ovulation induced in the hypophysectomized rabbit by anterior lobe extracts. By MARGARET HILL and A. S. PARKES.

Ovulation, which takes place in the rabbit only after copulation (Heape), is inhibited by the removal of the pituitary body within one hour after copulation (Fee and Parkes). To complete the evidence that ovulation depends on stimulation of the anterior pituitary body by the act of copulation, it was clearly necessary to induce ovulation by administration of extracts of the anterior lobe to rabbits hypophysectomized immediately after copulation.

As a preliminary, it was thought advisable to repeat the work of Bellerby and of Friedman, and test the extracts on intact non-copulated œstrous rabbits. Ovulation occurred freely in such injected animals, and incidentally opportunity was provided to study the subsequent history of the follicle induced to ovulate without copulation.

The difficulty of obtaining active protein-free extracts of ox pituitaries led to extensive trials being made of extracts of urine of pregnancy and of placentæ, both of which are thought to contain the active principle. The tissue extracts were made by the NaOH method described by Teel, while the urine preparations were made by alcohol fractionation of the alcohol insoluble portion of the concentrated urine. Hypophysectomy (by decerebration) was carried out as described previously (Fee and Parkes).

Thirty normal non-copulated œstrous rabbits were used in the preliminary work. In six the extract proved to be toxic, but ovulation was induced in eleven, as the result of a single injection. It was soon found that the follicles induced to ovulate formed corpora lutea. By autopsy, at various times up to 30 days after injection, it was shown that pseudo-pregnancy of normal duration, with the normal uterine and mammary changes, resulted from the induced ovulation. This result shows that the stimulus to luteinization is initiated as the result of ovulation and not as a delayed effect of copulation.

Forty rabbits hypophysectomized within half an hour of copulation were injected—intravenously in all the later experiments. Many of these died before the time for ovulation, owing to the toxicity of the extracts. Of those surviving for not less than 12 hours after copulation, sixteen receiving urine extracts (three subcutaneous, others intravenous), all failed to ovulate, but five out of seven receiving extracts of rabbit placentæ intravenously ovulated a total of twenty-six follicles. Animals receiving extracts of anterior lobe itself all died prematurely.

These results throw some doubt upon the nature of the gonad stimulating substance obtainable from urine of pregnancy, and emphasize the desirability of avoiding the current fallacy of testing such extracts on animals with their own hypophysis intact.

The effect of amino-acids on the rate of oxidation of adrenaline. By MARION O. P. WILTSHIRE.

It has been shown in a previous communication that various tissues and also blood serum markedly diminish the rate of destruction of adrenaline as tested on the jejunum of the rabbit. This occurs both with living and dead tissue (1). According to some authors, however, extracts of various organs of the body antagonize the inhibiting action of adrenaline on uterine and intestinal muscle (2).

It is possible that this effect of tissues may be due either to proteins or to amino-acids. Abderhalden and Gellhorn, from experiments on the cardiac muscle of the frog, state that the effect is due to amino-acids and not to colloidal material.

Abelous, Soulié and Toujan (3) investigated the action of putrefactive substances on adrenaline. They estimated, by chemical methods, the amount of adrenaline formed post-mortem in the suprarenals, and found that this was more than doubled by the presence of putrefactive

substances derived from the muscles. These results have been contested, but a partial explanation of them may be that the apparent increase in adrenaline content is due simply to the protection of the adrenaline from destruction by the amino-acids.

Experiments with glycine, alanine, *d*-alanine, *l*-tyrosine, and *l*-leucine show that while these substances have apparently no effect on the contractions of isolated gut in Ringer, when they are present the time required for the destruction of adrenaline is enormously prolonged. The amount of an amino-acid required to produce this effect is very much less than the total free amino-acid content of blood. A percentage concentration of 0·0009 of *l*-tyrosine will greatly prolong the time during which adrenaline is active. For example, an adrenaline solution 1 in 50 million, which had no action after 20 seconds, is found to cause inhibition after 7 minutes, that is, the rate of destruction of adrenaline is 21 times less. With larger amounts of amino-acids the effect is still more marked.

As far as the experiments have proceeded, it is found that the amino-acids are not all equally effective. The order of activity is *d*-alanine, alanine, *l*-tyrosine, *l*-leucine, glycine, the first being the most powerful.

In the case of *d*-alanine especially, the effect on adrenaline is very marked, and a further phenomenon is noticed. If the gut is exposed to *d*-alanine, concentration 0·0009 p.c., and then thoroughly washed with Ringer-Locke solution, the whole behaviour of the gut is altered. A concentration of adrenaline which before exerted no action after 1½ minutes is now found to cause marked inhibition after 30 minutes even though no alanine is present. The gut has apparently been sensitized by this amino-acid. So far this effect has not been noticed with *l*-tyrosine or any of the other amino-acids. It has been observed also that *l*-tyrosine does not make effective an amount of adrenaline which is in itself ineffective, but it may be that alanine does so.

1. M. O. P. Wiltshire. Proc. Physiological Society. Feb. 8th, 1930.
2. J. B. Collip. Amer. J. Physiol. 53. pp. 343 and 477. 1920.
3. Abelous, Soulié and Toujan. C. R. Soc. Biol. 57. ii. p. 589. 1905; 58. ii. p. 16. 1906.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY,
April 12, 1930.

The influence of experimental lesions of the spinal cord upon the knee-jerk and crossed extensor reflexes. By J. F. Fulton, E. G. T. Liddell and D. McK. Rioch. *Oxford.*

An attempt has been made to trace in the spinal cord the tracts responsible for the differences between "spinal" and "decerebrate" knee-jerks. Employing the technique of Ballif, Fulton and Liddell(1), with a new frictionless myograph(2), knee-jerks have been compared on the two sides in a series of 15 preparations with incomplete transverse lesions of the spinal cord (low dorsal level) made three weeks previously, and in another series in which similar lesions were made on the day of experiment. After such observations the spinal cord was transected completely, and the "spinal" jerks which resulted were compared (both sides) with those previously recorded. The crossed extensor reflexes were also studied before and after transection. Within two or three minutes after complete semisection the knee-jerks on the same side became spinal in character, *i.e.*, only the red fibres responded, giving a more prolonged jerk(3), and the responses were more readily inhibited by single break-shocks applied to an ipsilateral nerve. In a *chronic* semisected preparation, subsequent complete transection sometimes slightly increased the susceptibility to inhibition, indicating that the descending fibres from the opposite side may come to exert an appreciable influence upon the contralateral extensor centres. After acute semisections, subsequent complete transection has little or no effect upon the knee-jerk of the semisected side. Our attempts at more accurate localization of the descending tracts responsible for the differing susceptibility to inhibition suggest that they pass for the most part in the ventral quadrant of the spinal cord, since transection (acute and chronic) of the ipsilateral dorsal quadrant is without effect upon the jerk. It would appear, moreover, that the more important tracts controlling local inhibition descend in the medial part of the ventral quadrant. In chronic preparations extensive compensation occurs following any lesion wherever it may be situated,—a fact which makes the more precise localization of the tracts functioning in the normal

animal exceedingly difficult. In preparations in which the susceptibility of the knee-jerk to inhibition is great, the crossed extensor reflex is greatly depressed or absent. When the susceptibility of the jerk is less, the crossed reflex is generally proportionally greater. The degenerations following the surgical lesions have been studied in all cases by Marchi's method.

1. Ballif, L., Fulton, J. F., Liddell, E. G. T. Proc. Roy. Soc. B, 98. p. 589. 1925.
2. Eccles, J. C. and Sherrington, C. S. This Journ. 69. Proceedings, p. i. 1929.
3. Denny-Brown, D. Proc. Roy. Soc. B, 104. p. 371. 1929.

Sinus caroticus reflexes upon venous pressure, liver volume and heart volume. (*Preliminary communication.*) By C. Heymans and Jean J. Bouckaert.

It is a known fact that a diminution of arterial blood-pressure in the sinus caroticus, by clamping the common carotids, brings about reflexes of heart acceleration, arterial vasoconstriction and increased adrenalin output.

In order to contribute to the study of the influence of the sinus caroticus reflexes on the circulation, the following questions were examined: influences of lowering the blood-pressure in the sinus caroticus upon the cava and portal venous pressure, upon the liver volume, upon the heart ventricle and the heart stroke volume. The experiments were performed on dogs anæsthetized with chloralosane.

1. *Venous pressure.*

A. Vena cava inferior. The pressure in the central ends of the femoral or renal veins does not change during the arterial hypertension following the occlusion of the common carotids.

B. Venous pressure in the right auricle. The venous pressure measured in the right auricle is slightly increased, when the pressure in the sinus caroticus is lowered. This rise in venous pressure seems to be dependent on the reflex respiratory stimulation induced by clamping the common carotids; this is shown by the fact that, when the animal is under artificial respiration, the occlusion of the common carotids produces, instead of a rise, a fall of venous pressure in the right auricle.

C. Portal venous pressure. The portal venous pressure is markedly increased after occlusion of the common carotids; after opening them again it is decreased. The portal venous pressure thus reacts to the

occlusion of the common carotids in the same way as the general arterial blood-pressure.

2. *Liver volume.*

The liver volume is slightly decreased during the occlusion of the common carotids. This diminution of liver volume seems to be related to a reflex constriction of the liver arteries, since, after the liver arteries are tied, the occlusion of the common carotids produces, instead of a diminution, an increase of the liver volume.

3. *Heart volume and stroke volume.*

The volume curve of the ventricles of the normal and the denervated hearts, measured by a Henderson's cardiometer in dogs under artificial respiration, is markedly decreased by the occlusion of the common carotids. The heart stroke volume, under the same experimental conditions, is also diminished when the carotids are clamped and increased when they are opened again.

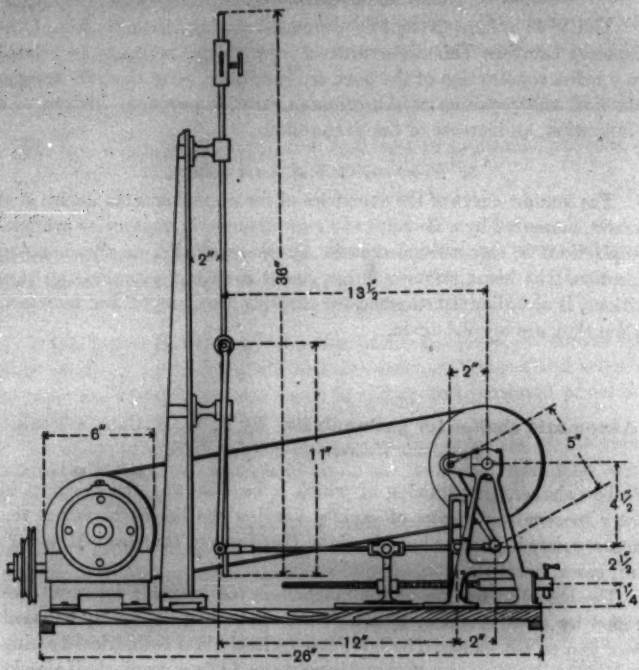
Automatic shaker for gas analysis. By E. W. H. CRUICKSHANK (*Dalhousie University, Halifax, N.S.*).

The shaker, the drawing of which is here shown, was designed in order to secure a means of rapidly varying the height to which the mercury rises in the gas burette of the Haldane or Haldane-Henderson gas apparatus.

The essential feature of the machine is the movable fulcrum, controlled by a worm screw, by which the stroke can be rapidly changed from two to ten inches. The lever moves freely in a cylindrical bearing at the fulcrum. In order that the lever actuated by a crank shaft should not change its position in the cylindrical bearing of the fulcrum during or after adjustment of the stroke, there are placed, one on each side of the lever, two guides, in which run pulleys attached to the ends of a short transverse bar fixed to the lever two inches from the connecting rod.

The means whereby a smooth movement is obtained of the distal end of the lever through the arc of a circle, which varies in size according to the position of the fulcrum, is sufficiently indicated in the drawing of the side elevation. To secure a movement of ten strokes per minute a suitable reducing gear is used. In the machine as at present used, the speed of the motor ($\frac{1}{4}$ H.P.) is 1250 r.p.m.; its speed reduction ratio

is 1 : 9; the reducing gear ratio is 1 : 14. By using step-down 6 in. pulleys on the machine a great range of speed can be obtained.



Automatic shaker for gas analysis.

The machine is noiseless, portable, and can be adapted to various uses. It was made in the machine shop of the department by Mr G. S. Sandoz, to whom I am indebted for the drawing which is made to one-eighth scale.

PROCEEDINGS
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May 10, 1930.

The œstrous cycle of the Hamadryas baboon. By S. ZUCKERMAN and A. S. PARKES.

The œstrous cycle of the baboon is characterized by enormous swelling of the vulva and perineum during the follicular phase. The enlargement, which begins at the end of the period of menstruation, reaches a climax at the time of ovulation and is obviously associated in the normal animal with the maturation of the Graafian follicle. The presence of this external sign of proœstrus and œstrus, together with the occurrence of true menstruation, greatly facilitates the analysis of the Primate menstrual cycle.

The length of the cycle and of its component parts varies from animal to animal, and also in the same animal from time to time. Thirty-six cycles observed in ten different baboons had an average length of 30 days, the variation being from 23–40 days. Menstruation lasts 3–4 days and the swelling of the vulva follows immediately. In about 5–7 days the swelling reaches its maximum and it remains fully developed for about a week. Ovulation then takes place and the swelling subsides completely in 2–3 days. Menstruation begins about 10 days later. The ease with which the time of ovulation can be established makes it possible to ascertain accurately the length of pregnancy. Calculated from the subsidence of the last swelling to the time of parturition, the average length of five pregnancies was 169 days, the range being 154–185 days. The cycle, as diagnosed by the external signs, is completely absent during pregnancy and lactation, and recommences 10–20 days after the cessation of suckling.

Unilateral ovariectomy does not alter any feature of the cycle, but double ovariectomy, of course, eliminates all the cyclic changes. The external genitalia atrophy to a point below the resting state of the normal cycle. Injection of œstrin into the ovariectomized baboon causes development of the vulval swelling identical with that found in the normal female. Very large amounts of œstrin are required, however, 15,000 mouse units per day for 4–5 days being necessary.

Sensory impulses in crustacean nerve. By T. CUNLIFFE BARNES[1] (*Cambridge*).

The primary object of the investigation was to find out if sense organs responding to pressure were present in the limbs of Arthropods which exhibit reactions to gravity.

The entire nerve from the meropodite of a walking limb in *Cancer pagurus* was connected by electrodes to an amplifier leading to a loud speaker or a Matthews' oscillograph. Ascending impulses were present in almost all preparations with absence of any stimulation and also descending impulses from the cut end of the nerve. A drying leg gave the most spontaneous discharges, but these were seldom entirely eliminated by irrigation with sea water or by immersion of the limb.

The individual impulses are easily detected in spite of the large size of the nerve, since they give potential changes of the order of 15–20 microvolts. They vary in size and probably in conduction rate. Records of the impulses which descend from the cut end of a nerve are shown in Fig. 1. They travel at about 5 metres a second, as shown by the form of the diphasic response, but they are probably travelling in motor fibres. Sensory impulses produced by joint movement or stimulation of hairs have about the same form, but the rate of conduction has not been determined.

The joint and also the substance in the dactylopodite contain sensory endings resembling those in the hairs described by Retzius. Bending the joint gives a discharge of impulses and bending the hairs gives a similar discharge (Fig. 1, *C*). The frequency in the whole nerve may reach 80 a second: the discharge only takes place during the actual movement of the joint and is proportional to the rapidity and extent of the movement.

The nerves to the rows of hairs on the dactylopodite are arranged in four chief strands, the fibres for the dorsal hairs being 3–7μ in diameter and those from the ventral and lateral 16–22μ. The large fibres give a louder response and the corresponding reflex is far more vigorous. There is a distinct reflex for each group of hairs, the limb moving in the direction of the stimulated group.

Cutting the nerve or pinching the proximal end produces a long outburst of descending impulses which may last 10 seconds or more and declines gradually in frequency (Fig. 1, *D*, *E*, *F*). An analysis of the rhythms shows that very few fibres contribute to the discharge and these

[1] Province of Quebec Scholar, Canada.

are probably motor, since no discharge is produced by cutting the sensory nerve where it leaves the dactylopodite. In Fig. 1, *F*, it will be seen that the rhythm may fall as low as 10 a second. A short period of contraction occurs when these discharges are produced, and on one occasion

Fig. 1.

A, Monophasic, and *B*, diphasic, impulses descending from the cut end of the nerve (probably in motor fibres—see text).

C. Discharge produced by moving hairs.

D, *E*, *F*. Discharge produced by cutting the nerve showing regular succession of impulses with declining frequency. *D*. 2nd second after cutting; frequency of regular series 92 per sec. *E*. At 3rd second, frequency 65 per sec. *F*. At 7th second, frequency 12·5 per sec.

a persistent tonus was found to be associated with a persistent discharge at 73 a second in one fibre of the nerve. Evidently these persistent discharges may play a large part in the peripheral tonus of Arthropod muscles.

My thanks are due to Prof. Adrian for many helpful suggestions.

A Brodie-Starling kymograph extension for doubling the recording surface. By I. DE BURGH DALY.

It is sometimes found that the recording drum (10 in. high) of the standard Brodie-Starling kymograph will not accommodate the desired number of writing levers without overlapping of the tracings. The photograph shows a simple method of doubling the recording surface, which does not necessitate extensive alterations to existing machines.

The parts required consist of

A. $\frac{7}{8}$ in. dia., 24 in. long, Bright Mild Steel, centred at each end.

B. $\frac{5}{8}$ in. dia., 24 in. long, B.M.S., centred at each end.

C and *D*. $\frac{3}{4}$ in. dia., 11 in. long, B.M.S. extension pieces to carry centres.

E and *F*. $1\frac{1}{4}$ in. dia., 3 in. long, B.M.S., $\frac{3}{4}$ in. hole through centre and four $\frac{1}{4}$ in. Whitworth set screws.

G. 1¼ in. dia., 6½ in. long, B.M.S., ⅜ in. hole through centre and two wing screws.

H. 1¼ in. dia., 11 in. long, steel drum, ends centred; this drum, supported by arms *I*, *I'* bolted to *G*, swings on arc of circle of 6 in. radius.

J, *J'*. Standard size drums (C. F. Palmer, Ltd.).

K. Extra pillar ⅜ in. dia. for carrying additional recorders.

The lower smoked paper is put on first and made taut in the usual way. The upper paper which is made 2 in. longer than the lower is then fitted and stretched by means of the jockey pulley (*H*). The shafts *A* and *B* carrying the drums do not require further support or a separating piece between their upper ends. The machine can be used as a single paper kymograph with the extension in position.

With the exception of the standard drums the parts were made in the laboratory workshop. C. F. Palmer, Ltd., 63a, Effra Road, London, S.W.2, is prepared to supply the complete extension.

Some effects of the administration of excessive doses of irradiated ergosterol to normal and to parathyroidectomized dogs.

By N. B. Taylor, H. D. Branion and H. D. Kay (*Toronto*).

Hess, Weinstock and Rivkin(1) have shown that vitamin D administered to dogs raises the serum calcium after this has been lowered by parathyroidectomy. Such is also the outstanding effect of the administration of parathyroid extract. Brougher(2) has reported that cod liver oil or viosterol is of definite value in preventing the onset of tetany in dogs after parathyroidectomy. This observer and others have also found that the tetany resulting from parathyroid removal in man is capable of control by irradiated ergosterol (viosterol). Finally there has come to our notice during the course of these experiments the work of Morgan and Garrison(3), who claim that irradiated ergosterol increases the susceptibility of dogs to the administration of parathyroid extract. All these facts strongly suggest an interdependence of the actions of the parathyroid hormone and vitamin D.

The experiments which we report furnish evidence, we believe, that the vitamin exerts its effect upon calcium metabolism through the stimulation of parathyroid function.

The experiments were carried out upon dogs and were planned with three objects in view. (1) To demonstrate the effects of vitamin D upon

the course of parathyroid tetany. (2) To compare the effects upon normal animals of vitamin excess with those resulting from parathyroid overdosage. (3) To determine the actions, if any, of vitamin overdosage in animals from which all parathyroid tissue had, so far as was possible, been removed.

(1) In this series the ordinary operation of thyro-parathyroidectomy was performed. A certain proportion of the animals developed violent tetany. The serum calcium fell to between 5 and 6 mgms. per 100 c.c. A dose of 1 c.c. of a special preparation of irradiated ergosterol, having 10,000 times the potency of cod liver oil (Mead, Johnson and Co.), when given by mouth restored the animals to normal within an hour.

(2) In this series of experiments ten normal animals were used. 0·14 c.c. per kilo of the same preparation of irradiated ergosterol was given by mouth twice daily. Death occurred in from 60 to 84 hours. The symptoms, which made their appearance on the second day, were depression, vomiting—often of blood or bloody fluid, and the passage of blood-stained stools. The serum calcium was raised to between 16 and 20 mgms. per 100 c.c. The post-mortem findings were invariably the same, namely, intense congestion of, with extravasation of blood into the gastric mucosa, and hæmorrhages into the cavity of the stomach and upper intestine. In severe cases the gastric mucosa was a deep chocolate colour, resembling liver tissue. The symptoms, the hypercalcæmia and the post-mortem findings are identical with the effects of parathyroid overdosage as reported by Collip *et al.*(4) and Macleod and Taylor(5).

(3) Animals from which the parathyroids had been removed were found to show a certain resistance to overdosage. When the ordinary operation of parathyroidectomy was performed the survival period was from 168 to 212 hours. In those animals in which a free dissection of the neck was made from the level of the hyoid to the sternum, and all sites that might harbour parathyroid tissue carefully cleaned, the resistance to overdosage was great. All animals eventually succumbed, but the survival time was from 300 to 360 hours. Irradiated ergosterol was also much less effective in relieving these animals of tetanic symptoms. Low calcium, twitchings, trismus, etc. persisted for days though ergosterol in large doses was being administered. When the tetany was severe, maximal doses failed to cure the condition and the animal died in convulsions. This contrasted strongly with the rapid cure which followed the administration of a single dose to an animal upon which the usual thyro-parathyroidectomy had been performed. In none of these experiments was calcification of the arterial walls detected.

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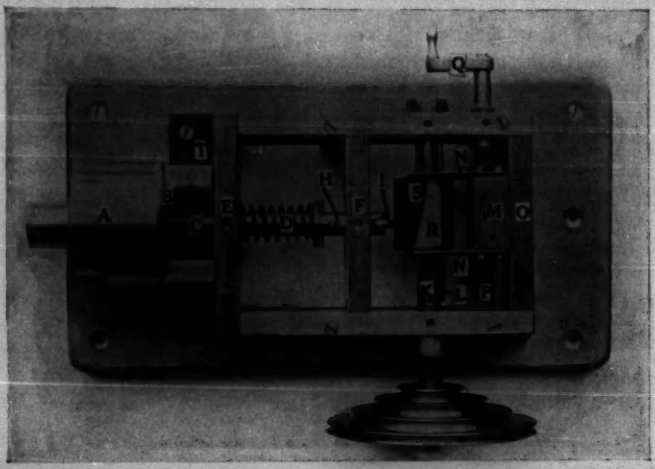
A variable cam poppet valve for negative pressure respiration.

By I. DE BURGH DALY and W. V. THORPE.

Entire animals or isolated perfused lungs have been placed in an air-tight chamber with the trachea connected to the outside air, ventilation



of the lungs being brought about by rhythmic variations in the chamber pressure. Various methods have been used for producing the air pressure changes (1, 2, 3, 4, 5). The device in use at present consists of a Cenco exhauster which continuously removes air from the chamber with a consequent reduction of the chamber pressure and inflation of the lungs. A valve fitted to the wall of the chamber opens periodically to admit air, thus raising the pressure and causing collapse of the lungs by virtue of their own elasticity. On closure of the valve inflation of the lungs again

occurs. The valve is operated by a variable cam which permits of the inspiratory depth and the relative durations of the inspiratory and expiratory phases to be varied without alterations in the respiratory frequency.

The respiration chamber is connected to the cylindrical box *A*, in which is seated the valve head. This is a steel disc $\frac{5}{16}$″ thick fitted with a fibre or leather washer to seat on the 1½″ hole in the box at *B*. The end *C* of the valve stem (¼″ diam.) is threaded (12 T.P.I.) and the head is located on this by two nuts, thus enabling adjustment of the valve clearance. The valve head is held against its seating by a suitable helical spring *D*. The valve stem works in the bearings *E*, *F*, located in the main frame. At *F* the valve stem is fitted with a short ⅛″ square sunk key—in order to maintain the correct position of the soft steel roller *I* which engages with the cam. The cam slides upon and is driven by the ½″ shaft *K* fitted with a sunk key (⅛″ square) *L*. The position of the cam on this shaft is governed by the slide *M* which engages with the cam through the arms *N*, *N*. The slide bears on the face *O* of the main framework and the threaded (16 T.P.I.) ⅜″ shaft *P*, and is actuated by the handle *Q*. The cam is made in two parts; a cylinder *R*, 1″ diam. and 1½″ long, sliding on the keyed shaft, and the case-hardened cam piece *S*, which is bolted to the cylinder. Cam pieces are cut from steel tubing of 1″ bore and ⅛″ to $\frac{5}{16}$″ wall, bolted to a case-hardened cylinder similar to *R* and the edges filed so as to form the tangent of the cylinder. The cam piece is then case-hardened and bolted to *R*. Several cams can be made for the same cylinder. That illustrated is tapered from 20° to 90°. The frame consists of steel bars (1″ by ¼″ section) mounted on 1″ pillars on the base plate *T* of ¼″ iron.

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The carotid sinus. By E. VOLHARD.

The slowing of the heart which follows a rise of the cerebral blood-pressure is attributed by H. E. Hering and by C. Heymans to a reflex originating in the carotid sinus. Since, in their experiments, the effect completely disappeared after denervation of the sinus, they consider the sinus reflex to be the only mechanism on which the cerebral regulation of the heart rate depends.

At the suggestion of Dr G. V. Anrep I undertook a reinvestigation of this question, using the technique of the innervated heart-lung preparation in which the head was perfused through the internal carotid and the vertebral arteries. The carotid sinus on both sides was completely denervated and excluded from the circulation.

Under these conditions changes in the cerebral blood-pressure continue to affect the heart rate; a rise producing a slowing and a fall an acceleration of the heart. However, the presence of this effect depends on various conditions over which we have not as yet complete control. Injection of adrenaline or of pituitary extract into the cerebral circulation also produces a very considerable slowing of the heart rate. This slowing is more pronounced than that following a purely mechanical rise of the blood-pressure to the same height as produced by the vaso-constrictor substances. The slowing of the heart cannot be due to a partial anæmia of the brain, for if the blood-pressure in the head is raised during the adrenaline effect the heart slows still further, and if the blood-pressure is dropped the heart accelerates although the blood supply must be worse than before. Moreover, direct measurements of the blood flow through the head failed to show an appreciable diminution of the blood flow during the adrenaline vaso-constriction.

I am as yet unable to state the exact mechanism on which all these effects depend. It is possible that a part of the vertebral arteries or the circle of Willis and its branches may play a rôle in the regulation of the heart rate. A direct central effect is not excluded.

The analysis of these facts is being continued. At present, however, I feel justified in concluding that the cephalic control of the heart rate does not depend exclusively on the carotid sinus.